HISTOCHEMICAL OBSERVATIONS IN PRECLINICAL MOUSE MUSCULAR DYSTROPHY

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In a previous investigation of the preclinical histopathology of mouse muscular dystrophy, 2 kinds of litters were studied: (1) offspring consisting entirely of dystrophic (*dy/dy*) mice obtained by artificially inseminating homozygous F_1 hybrid females derived from 129/Re-*dy*/+ × C57BL/6J-*dy*/+ matings with spermatozoa from homozygous F_1 hybrid males of the same genotype,[1] and (2) offspring from dystrophic females inseminated with mixtures of genetically tagged spermatozoa utilizing the eye-color markers: pink-eyed dilution (*p*) and its wild-type allele (+) for identification of dystrophic (*p/p dy/dy*) and heterozygous mice (+/+ *dy*/+).[2,3] The 2 types of offspring permit both between-litter (dystrophic-normal) and within-litter (dystrophic-heterozygous) histopathologic comparisons of muscle and other tissues in preclinical stages. Pink-eyed and black-eyed individuals can be identified by Day 12 of gestation. Although offspring from crosses of F_1 hybrids (129B6) are homozygous for dystrophy (*dy/dy*), they are otherwise a genetically segregating population. Variation in genetic background does not, however, cause appreciable differences in the expression of *dy/dy* genotype,[3] and F_1 hybrids were used for breeding because of their increased vigor and longevity.

Previous to the development of all-dystrophic litters, studies on the pathogenesis of muscular dystrophy were not practical because dystrophic males do not breed and dystrophic females have only a limited reproductive life. In litters of 129/Re-*dy*/+ × *dy*/+ mated naturally, only a maximum of 25% are dystrophic and the dystrophic animals cannot be identified until clinical signs appear at about 10–14 days of postnatal life. Also, by this mating, heterozygotes cannot be recognized and only limited biopsy tissues can be obtained during the preclinical period.[4] Obviously, by the time symptoms appear, muscle metabolism is deranged in numerous ways and many alterations observed are the result, rather than the causes(s), of the basic defect.

Through the study of tissues from the stages preceding clinical signs and symptoms, it is hoped that the primary abnormality may be uncov-

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TABLE I

GENOTYPES OF PARENTS AND OFFSPRING

Females	*Males, sperm*	*Offspring*	
p/p dy/dy	*p/+ dy/dy*	*p/+ dy/dy*	black-eyed
		p/p dy/dy	pink-eyed, dystrophic
p/p +/+	+/+ +/+	*p/+ +/+*	
		+/+ +/+	black-eyed, normal
p/p dy/dy	*p/p dy/dy* plus +/+ +/+	*p/p dy/dy*	pink-eyed, dystrophic
		p/+ dy/+	black-eyed, heterozygus

ered. The most promising approach is that of retrograde analysis, progressing toward the younger stages in an animal's life, to determine the time of earliest disease onset, manifestations, and occurrence. The present paper describes histochemical findings in preclinical dystrophy particularly as relating to (1) the differential time involvement of skeletal muscle, and (2) the selective nature of the disease in muscle bundles of specific fibers and of fiber segments.

MATERIALS AND METHODS

Animals. Mixed and all-dystrophic litters of mice were produced as previously described.[2,5] The genotypes of parents and offspring are listed in Table I. All mice were hybrids (129B6) derived from crosses between strains 129/Re and C57BL/6J, carrying *dy* and *p* as shown. Although it would have been ideal to use *p/p* females in all cases, they were not available in the quantity needed so that *p/+* females were employed as well.

The offspring were delivered by cesarean section starting with Day 18 after artificial insemination and were fostered on 129B6F$_1$ or C57BL/6J mothers. On Days 1, 3, 6, 14, and 26 of postnatal life 5 animals were sacrificed. The muscles surrounding the femor (musculus vastus lateralis) were removed and processed according to the requirements of the staining methods used.

In a special series, the histochemical differentiation of developing muscle fibers in 129B6—*p/p dy/dy* and +/++/+—was studied from the Day 15 of gestation to 2 weeks after birth. Muscles analyzed were the femoral complex, heart, and tongue. Three to 6 animals of each genotype were sacrificed each day.

Histochemical procedures. Tissue blocks of approximately 2 x 4 mm. were placed on a microtome chuck and immersed in liquid nitrogen for about 10 sec. Sections were cut at 10 μ on a cryostat maintained at $-16°$ to $-20°$C, then placed on coverslips and immersed in incubation media according to procedural requirements, fixed for 10 min. in 10% formol-saline, counterstained with hematoxylin eosin if deemed necessary, and mounted in glycerin jelly. Control sections were treated identically, but incubated in media without substrate.

Phosphorylase was stained according to the method of Takeuchi and Kuriaki [6] as

modified by Eränkö and Palkama.[7] The modification was distinctly superior to the original method; apparently, the high viscosity of the incubation mixture from polyvinylpyrrolidone and high substrate concentration prevented diffusion of the reaction product, thereby improving sensitivity and localization of the reaction. Cholinesterase was localized according to Koelle and Friedenwald,[8] as modified by Couteaux.[9] Lactic dehydrogenase (LDH) was localized by the methods of Hess *et al.*,[10] and Fahimi and Amarasingham,[11] except that in the latter procedure phenazine methosulfate was replaced by 2-methyl-1, 4-naphthoquinone (menadione). It was found that like phenazine methosulfate, menadione, used as an intermediate electron carrier, made the staining system independent of tissue nicotinamide adenine dinucleotide-diaphorase (NADH-diaphorase).

NADH-diaphorase was demonstrated by the procedure of Nachlas *et al.*[12] Alpha-glycerophosphate dehydrogenase (α-GPDH) was localized by the methods of Chason and Pearse [13] and Adams *et al.*[14] The first procedure utilizes phenazine methosulfate and the second, menadione, as intermediate electron carriers. The procedure of Adams *et al.* was also used for the localization of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) upon substituting DL-glyceraldehyde-3-phosphoric acid (GAP) for sodium glycerophosphate (GP) in the incubation mixture. If, instead of the free acid solution (Sigma Chemical Co., St. Louis, Mo.) which is essentially barium- and ethanol-free, the monobarium salt of GAP was used, it became necessary, because of insolubility of the barium salt, to convert it to the sodium salt. Following addition of 1 M HCl to barium GP, the precipitate is removed by centrifugation and the pH of the supernatant readjusted with M/15 Na_2HPO_4.

Results

Phosphorylase

In developing leg muscle, a positive phosphorylase reaction appeared in the large myotubes denoted as primary types, on Days 15 to 16 of gestation. Smaller secondary fibers with weaker-staining intensity occurred close to the primary fibers on fetal Days 17 to 18. Tertiary fibers giving little phosphorylase reaction developed on Days 19 to 20 of gestation (Fig. 1A). These 3 fiber types differentiated and developed as distinct populations, forming a quite regular checkerboard pattern which persisted throughout the postnatal period. In dystrophic mice, the earliest pathologic changes were detected at Days 19 to 20 of fetal life, coincident with and following the development of tertiary fibers. Tertiary fibers were involved first and in the absence of any detectable changes in primary and secondary fibers. Involvement of tertiary fibers, on Days 19 and 20, was not uniform, nor did it occur in all of them at once. Normal tertiary fibers could be recognized and they persisted in some areas of a muscle bundle whereas in others, tertiary fibers were still developing. Thus, tertiary-fiber involvement was discrete and strictly focal or multifocal within a muscle bundle. The earliest signs of abnormality were fiber contraction, disruption of myofibrils, and loss of phosphorylase activity. Typically, alterations in enzyme activity did not involve an entire fiber, but losses of phosphorylase occurred segmentally (Fib. 1B). Enzyme-free foci either involved the entire fiber width, making for a frag-

mented and striated appearance, or affected either central or peripheral portions of a fiber, giving it a moth-eaten appearance. Many of the necrotic segments contained quantities of glycogen granules located mostly within "central cores" formed by disrupted myofibrils.

On subsequent days, beginning with Day 1, secondary as well as primary fibers revealed dystrophic changes. Prior to coagulation necrosis, the primary fibers, which stained heavily for phosphorylase and strongly "red" and granular with eosin, underwent atrophy, whereas the less-eosinophilic "white" agranular fibers became hypertrophic. Consistently, fiber atrophy was associated with loss of phosphorylase, so that with minor variations, most fibers tended to react uniformly for phosphorylase. Thus, in dystrophic foci the normal checkerboard pattern was disrupted by the presence of coagulated fibers, the uniformity of fibers in enzyme reaction, and, in some instances, even in a seeming reversal, with respect to size and enzyme activity, of persisting fibers. The normal pattern remained and persisted in as yet unaffected areas of musculature of dystrophic mice. Even in dystrophic foci, apparently normal fibers were observed adjacent to partially or completely coagulated ones.

In contrast to skeletal muscle, the heart was entirely phosphorylase-negative. In the tongues of 26-day-old mice with early dystrophic foci, glycogen accumulations were more globular than granular as compared with those of the leg muscles.

NADH-diaphorase

In normal muscle, 2 kinds of fibers were easily distinguishable: an intensely staining type and another that stained less heavily (Fig. 2A and B). High enzyme activity occurred in the red fibers and little in the white fibers. Typically, no reaction was elicited when NADH was absent from the incubation mixture, but neither was activity visible upon omission of lactate. A uniformly faint reaction was obtained when LDH was omitted making distinction between the 2 fiber types no longer possible.

In dystrophic muscle, distinction between the 2 basic types of fibers was often difficult to make. For example, in fibers undergoing dystrophic atrophy and coagulation necrosis, NADH-diaphorase activity appeared heightened, and accumulated as clumps (Fig. 2C and D). These fibers showed activity even when NADH was absent from the incubation medium. As fibers underwent dystrophic hypertrophy, atrophy, or both, a correlation between diaphorase activity and fiber diameter no longer existed. More commonly, the difference in NADH-diaphorase between fibers was minor and the 2 basic fiber types could not clearly be distinguished.

Lactic Dehydrogenase

In LDH preparations of muscle from postnatal animals 2 types of fibers were demonstrable in both normal and dystrophic mice if menadione was absent from the incubation mixture. Fibers that stained heavily for LDH corresponded to the red fibers and those that remained virtually unstained, to the white fibers. In the presence of menadione all fibers stained prominently and uniformly for LDH, indicating that menadione had substituted for a low endogenous NADH-diaphorase content of white fibers. A fine reticular network of LDH activity occurred throughout the sarcoplasm in normal fibers, whereas a prominent granular pattern was present in fibers undergoing dystrophic changes. These changes consisted of atrophy or, more commonly, hypertrophy and central location of nuclei, and apparently involved the white fibers first, since the red fibers appeared normal. Coagulated fibers totally lacked LDH activity whereas some activity was still present even in fibers about to undergo coagulation necrosis.

α-GP and GAP Dehydrogenase

The procedure of Chason and Pearse[13] stained all muscle fibers weakly in both normal and dystrophic mice at 6 days of age (Fig. 3A and B). Uniformity of staining may be a result of the presence of phenazine methosulfate and nicotinamide in the medium, making the system independent of endogenous NADH-diaphorase. However, this possibility was not verified by preparation of reaction media omitting these substances. Even dystrophic fibers with centrally located nuclei had a faint but uniform reticular-reaction pattern. Only when the fibers had undergone coagulation necrosis was enzyme activity lost. In 14-day-old mice, staining had become deeper, but again was of similar intensity in normal and dystrophic mice. At 14 days of age dystrophic mice had extensive fibrotic lesions and muscle replacement so that the number of fibers per microscopic field was much reduced over that in normal mice.

The procedure of Adams *et al.*,[14] employing menadione as an electron mediator to provide for independency of the reaction from NADH-diaphorase, also gave a uniform staining reaction in all muscles. Enzyme activity was weak at 6 days of age and somewhat stronger at 14 days. A definite reaction occurred even in those fibers of dystrophic mice with centrally located nuclei. The staining reaction for α-GPDH was weaker at all ages than that for GAPDH in both normal and dystrophic mice (Fig. 4A and B). Absence of α-GP and GAP, respectively, from the medium gave no reaction in normal mice. However, with α-GP lacking, certain fibers of dystrophic mice, especially the large white fibers in or around a dystrophic focus, stained faintly but definitely.

Cholinesterase

In dystrophic foci a positive cholinesterase reaction occurred even at sites of myofibrillar disruption which is probably the very earliest light-microscopic lesion. Sections previously stained for cholinesterase were impregnated to show peripheral nerves, axons, and motor endplates. Myofibrils were heavily argentophilic. At sites of cholinesterase activity, nerve endings and endplates were structurally normal showing the usual ramifications, whereas in those regions where the nerve fibers were uneven and beady, cholinesterase was decreased or absent. Since cholinesterase activity persisted beyond myofibrillar disruption, structural and functional destruction of motor endplates was presumably brought about by coagulation necrosis of muscle fibers.

General Observations

The most outstanding histochemical feature of dystrophic muscle was the tendency toward uniformity in enzyme reactivity so that different fiber types could not easily be distinguished. Also, morphologic characteristics such as size difference in fibers were obliterated, at least during the advanced stages of the disease. Whereas mildly affected muscle tended to be homogeneous with respect to both structural and biochemical reactivity, in severely affected muscles, enzyme activity and fiber diameter were often the reverse of normal. This situation was brought about by losses in enzyme activity and hypertrophy or atrophy of dystrophic muscle. However, both structural and enzymatic heterogeneity of muscle fibers existed in mice with severe clinical signs of dystrophy, both in areas of apparent normality and in affected muscle complexes.

One of the first indications of dystrophic fiber atrophy was a serrated outline (as if the fiber had contracted). As the serrations became deeper, longitudinally sectioned fibers appeared segmentally constricted. The sites of constriction had the highest enzyme activity. In these heavily staining segments, longitudinal ridges developed, each ridge being separated from adjacent ridges by narrow furrows. This segmental staining was especially prominent in NADH-diaphorase and GAPDH preparations. Normal activity for all enzymes studied was present even in fibers with centrally located nuclei and those showing nuclear rowing; however, segmental-coagulation necrosis abolished all enzyme activity, leaving enzyme-free spots. Between the spots, enzyme activity was nearly normal or even increased (Fig. 5A, B, C, and D).

Discussion

With respect to staining intensity, the phosphorylase reaction revealed a heterogeneity of developing skeletal muscle. Fiber types differentiated

between Day 16 and Day 20 of gestation so that from fetal Day 20, 3 kinds could be distinguished, i.e., a heavily staining one, an intermediate kind, and one with little phosphorylase activity. The time course of development in $129B6F_1$ was analogous to that reported by Wirsén and Larsson[15] in A/J and CBA/J mice. It is likely that the 3 fiber types represent different fiber populations, each with its own inherent metabolic qualities.

Earliest dystrophic changes were observed at fetal Days 19 and 20. The fact that (1) they coincided with and immediately followed the development of the third fiber type, (2) fewer phosphorylase-positive fibers per microscopic field remained in dystrophic than normal fetal mice, and (3) the remaining fibers were either primary or secondary fibers, strongly suggests that dystrophy affects tertiary fibers first. Indeed, in and around dystrophic foci the normally quite regular checkerboard pattern was imperfect due to the preponderance among healthy fibers of deeply staining primary types, whereas in apparently normal areas, the checkerboard picture was preserved.

In LDH preparations, the small fibers had large amounts of LDH and the large fibers seemed to have little or no enzyme, except when an electron carrier has been added to the incubation medium.[16] Therefore, a reciprocal relationship appears to exist between LDH and phorphorylase with respect to small and large fibers. Similar observations were previously made in mice by Fennel and West,[17] and in man, rats, and pigeons by Dubowitz and Pearse.[18] However, the conclusion drawn by these authors that white muscle has low LDH and α-GPDH activity seems to be erroneous, since in white fibers, dehydrogenase activity depends on intracellular diaphorases. If however, the staining system is made independent of NADH-diaphorase by adding menadione or phenazine methosulfate to the incubation medium, high LDH and α-GPDH activities appeared in white muscle fibers. Such observations on the essential role of phenazine methosulfate in transferring electrons from NADH to nitro blue tetrazolium, and in demonstrating LDH in white muscle have previously been made.[9,13,19] Furthermore, quantitative estimations reveal that the white muscle of guinea pigs contains more than twice the amount of LDH and α-GPDH activity of red muscle.[20]

In NADH-diaphorase preparations, the small fibers had high activity and the large fibers low activity—confirming the conclusion reached above regarding the distribution of LDH.

In early postnatal dystrophy, white fibers with low NADH-diaphorase activity were involved first. Upon atrophy and contraction prior to coagulation necrosis, NADH-diaphorase seemingly became elevated, accumulating as large globular aggregations. This apparent increase in enzyme perhaps resulted from induction by excessive substrate, NADH.

NADH is formed upon oxidation of glyceraldehyde-3-phosphate by GAPDH. In the presence of adequate α-GPDH, NADH reduces dihydroxyacetone to form α-glycerophosphate, and the α-glycerophosphate so formed is then reoxidized by the intramitochondrial cytochrome c-linked α-GPDH to again produce dihydroxyacetone phosphate and reduced cytochrome c. A deficiency of either α-GPDH, GAPDH, or both would interfere with this proposed "shuttle" system whereby electrons can be carried from extramitochondrial NADH to the intramitochondrial electron transport chain.[21] A defect in this system might not only depress energy production in dystrophic muscle, but could partially block glycolysis by permitting NADH to accumulate.[22] Indeed, Coleman has found that triose phosphates do accumulate in dystrophic muscle because of decreased activity of both α-GPDH and GAPDH.[22,23] At 2½ weeks of age α-GPDH activity was only 75%, and at 10 days GAPDH was only 71% of normal, already in preclinical stages. A reduction of α-GPDH and LDH had previously been reported by McCaman; she also determined that the reduced enzyme activities were coupled with increases in enzymes of the pentose shunt, through which dystrophic mice were able to maintain normal CO_2 production.[24,25] However, histochemical observations presented in this paper indicate that both enzymes, α-GPDH and GAPDH, are present in normal amounts, even in fibers undergoing early dystrophic changes. This discrepancy between quantitative and histochemical findings can be explained by the fact that at the time the biochemical studies were performed in mice 10 days and older, muscle damage due to dystrophy was already extensive and considerably fewer muscle fibers were present per unit tissue in dystrophic compared to normal muscle. Indeed, the presence of α-GPDH in white muscle as well as red suggests that the α-glycerophosphate shuttle, which has been postulated as a mechanism for coupling the oxidative reaction of glycolysis with the mitochondrial respiratory reactions, is operative in these fibers. However, in dystrophic fibers the shuttle might operate below normal, since α-glycerophosphate seemed to accumulate—i.e., in incubation mixtures without exogenous substrate, a faintly positive reaction for α-GPDH still appeared.

Since α-glycerophosphate occupies a central role in phosphatide biosynthesis, especially with regard to phosphatidic acid and inositol phosphatides,[26] and since it is well known that as muscular dystrophy progresses, the tissue spaces between remaining muscle fibers become occupied by fat cells,[27] * a tentative explanation of the origin of fat cells may be derived. Obviously these changes represent consecutive events in muscular dystrophy.

* There is considerably less fatty replacement of muscle fibers in mouse compared with human (pseudohypertrophic) muscular dystrophy.

We have also found that in affected fiber segments nerve endings and motor-end plates were functionally normal, since a positive cholinesterase reaction occurred even at sites of myofibrillar disruption. Presumably, structural destruction and nonfunction are brought about by coagulation necrosis, a process occurring within a muscle fiber and being caused by metabolic aberration(s) that are likely the primary effect of the mutant gene.

Summary

Histochemical procedures were used for the identification and localization of several oxidative and hydrolytic enzymes in preclinical hereditary muscular dystrophy of mice. Enzymes studied were phosphorylase, LDH, NADH-diaphorase, α-GPDH, GAPDH, and cholinesterase. The following observations were made.

A reciprocal relationship is found in red and white muscle fibers between phosphorylase activity on one hand, and LDH and NADH-diaphorase activities on the other. White fibers low in NADH-activity are correspondingly low in LDH, but high in phosphorylase. The converse situation pertains to the small red fibers. In LDH stains prepared by addition to the incubation medium of the electron mediator, menadione, all fibers contain LDH, indicating that menadione substitutes for the low endogenous content of NADH-diaphorase in white fibers.

Earliest dystrophic changes were observed on Fetal Days 19 and 20. Apparently, tertiary phosphorylase fibers are affected first. This observation is based on the appearance of dystrophic lesions coinciding with the development of the third fiber type, fewer phosphorylase-positive fibers per microscopic field remaining in dystrophic than in normal muscle, and the remaining fibers being either primary or secondary fibers.

Postnatally, the normal reciprocal relationship between phosphorylase and oxidative enzymes disappeared and there is a lack of correlation between fiber size and enzyme content. White fibers are low in NADH-diaphorase and stain for LDH only in the presence of menadione. LDH and NADH-diaphorase are present in affected segments of white fibers even at a stage when their nuclei are centrally located and extend in rows. Following hypertrophy in those fibers undergoing severe atrophic changes, NADH-activity is seemingly heightened, perhaps due to excessive NADH.

Activities of α-GPDH and GAPDH are comparable in early dystrophic and normal fibers.

Cholinesterase activity occurs in dystrophic fibers even at sites of myofibrillar disruption.

Fiber destruction seems to be caused by a process occurring within

muscle fiber rather than as a consequence of neuromuscular disturbance. This observation is based on the facts that skeletal muscle is affected differentially, i.e., only certain fibers of a muscle are involved, and initially, only certain segments are affected.

References

1. Russell, E. S., Silvers, W. K., Loosli, R., Wolfe, H. G., and Southard, J. L. new genetically homogeneous background for dystrophic mice and their normal counterparts. *Science 135:*1061–1062, 1962.
2. Southard, J. L., Wolfe, H. G., and Russell, E. S. Artificial insemination of dystrophic mice with mixtures of spermatozoa. *Nature 208:*1126–1127, 1965.
3. Meier, H., West, W. T., and Hoag, W. G. Preclinical histopathology of mouse muscular dystrophy. *Arch Pathol 80:*165–170, 1965.
4. Platzer, A. C., and Chase, W. H. Histologic alterations in preclinical mouse muscular dystrophy. *Amer J Path 44:*931–946, 1964.
5. Wolfe, H. G., and Southard, J. L. Production of all-dystrophic litters of mice by artificial insemination. *Proc Soc Exp Biol Med 109:*630–633, 1962.
6. Takeuchi, T., and Kuraiki, H. Histochemical detection of phosphorylase in animal tissues. *J Histochem Cytochem 3:*153–160, 1955.
7. Eränkö, O., and Palkama, A. Improved localization of phosphorylase by the use of polyvinyl pyrrolidone and high substrate concentration. *J Histochem Cytochem 9:*585, 1961.
8. Koelle, G. B., and Friedenwald, J. S. Histochemical method for localizing cholinesterase activity. *Proc Soc Exp Biol Med 70:*617–622, 1949.
9. Couteaux, R. Remarques sur les méthodes actuelles de détection histochimique des activités cholinesterasiques. *Arch Int Physiol 59:*526–537, 1951.
10. Hess, R., Scapelli, D. G., and Pearse, A.G.E. The cytochemical localization of oxidative enzymes. II. Pyridine nucleotide-linked dehydrogenases. *J Biophys & Biochem Cytol 4:*753–760, 1958.
11. Fahimi, H. D., and Amarasingham, C. R. Cytochemical localization of lactic dehydrogenase in white skeletal muscle. *J Cell Biol 22:*29–48, 1964.
12. Nachlas, M. M., Walker, W. G., and Seligman, A. M. A histochemical method for the demonstration of a phosphopyridine nucleotide diaphorase. *J Biophys & Biochem Cytol 4:*29–38, 1950.
13. Chason, J. L., and Pearse, A.G.E. Phenazine methosulfate and nicotinamide in the histochemical demonstration of dehydrogenases in rat brain. *J Neurochem 6:*259–266, 1961.
14. Adams, C.W.M., Davison, A. M., and Gregson, N. A. Enzyme inactivity of myelin: histochemical and biochemical evidence. *J Neurochem 10:*383–395, 1963.
15. Wirsén, C., and Larsson, K. S. Histochemical differentiation of skeletal muscle in foetal and newborn mice. *J Embryol Exp Morph 12:*759–767, 1964.
16. Fahimi, H. D., and Roy, P. Cytochemical localization of lactate dehydrogenase in muscular dystrophy of the mouse. *Science 152:*1761–1763, 1966.
17. Fennel, R. A., and West, W. T. Oxidative and hydrolytic enzymes of homozygous dystrophic and heterozygous muscle of the house mouse. *J Histochem Cytochem 11:*374–382, 1963.
18. Dubowitz, V., and Pearse, A.G.E. A comparative study of oxidative enzyme and phosphorylase activity of skeletal muscle. *Histochemie 2:*105–117, 1960.

19. VAN WIJHE, M., BLANCHAER, M. C., and JACYK, W. R. The oxidation of lactate and α-glycerophosphate by red and white skeletal muscle. II. Histochemical studies. *J Histochem Cytochem 11:*505–510, 1963.

20. BLANCHAER, M. D., VAN WIJHE, M., and MOZERSKY, D. The oxidation of lactate and α-glycerophosphate by red and white skeletal muscle. I. Quantitative studies. *J Histochem Cytochem 11:*500–504, 1963.

21. BOXER, G. E., and DEVLIN, T. M. Pathways of intracellular hydrogen transport. *Science 134:*1495–1501, 1961.

22. COLEMAN, D. L. Accumulation of triose phosphates in dystrophic muscle homogenates. *Arch Biochem Biophys 111:*494–498, 1965.

23. COLEMAN, D. L. Studies on the acetoacetate-like compound found in dystrophic muscle homogenates. *Arch Biochem Biophys 111:*489–493, 1965.

24. MCCAMAN, M. W. Dehydrogenase activities in dystrophic mice. *Science 132:* 621–622, 1960.

25. MCCAMAN, M.W. Enzyme studies of skeletal muscle in mice with hereditary muscular dystrophy. *Amer J Physiol 250:*897–901, 1963.

26. KANFER, J., and KENNEDY, E. P. Metabolism and function of bacterial lipids. II. Biosynthesis of phospholipids in *Escherichia coli. J Biol Chem 239:*1720–1726, 1964.

27. WEST, W. T., and MURPHY, E. D. Histopathology of hereditary progressive muscular dystrophy in inbred 129 mice. *Anat Rec 177:*279–295, 1960.

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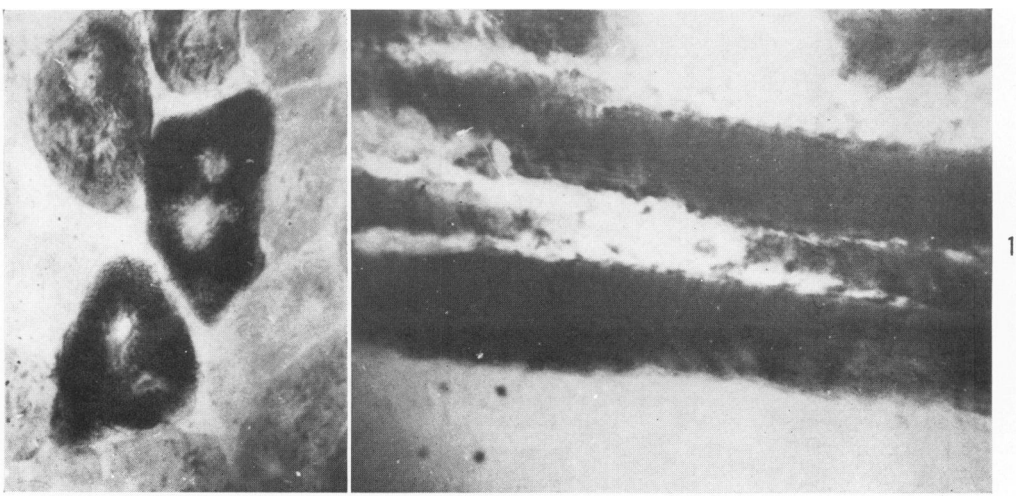
[*Illustrations follow*]

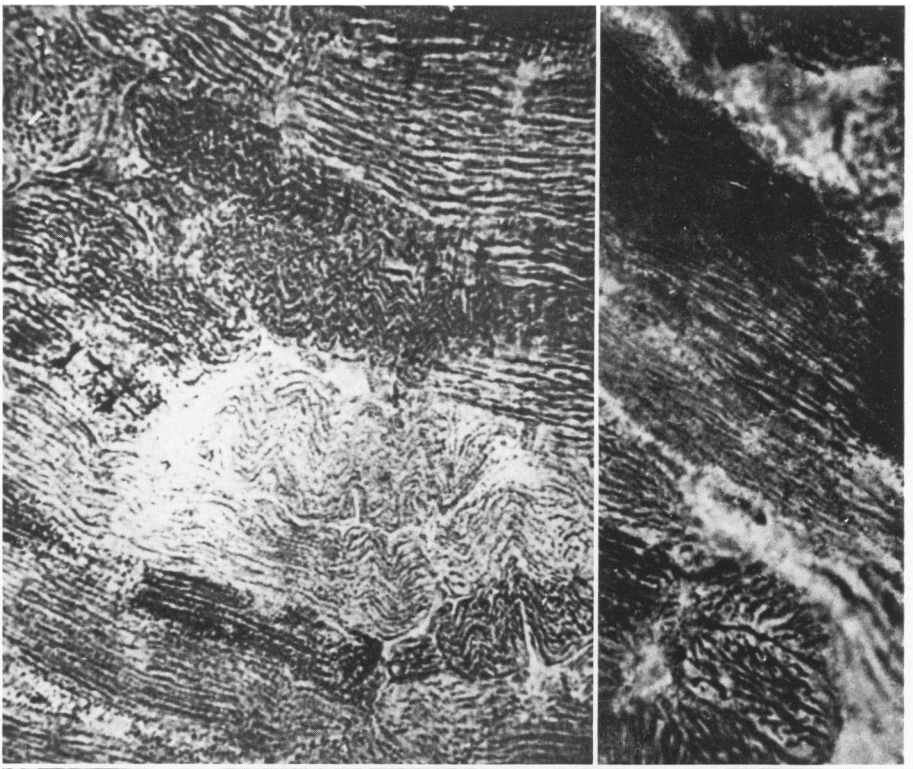
Legends for Figures

All sections are of muscle fiber from mice of ages indicated.

Fig. 1. Fetal Day 20. A. Phosphorylase-staining intensity of fibers: primary, darkly stained; secondary, lighter; tertiary, very weak. × 675. B. Early dystrophy; only certain fibers of bundle involved, and not always entire fiber. Segmentally, enzyme-free spots (phosphorylase loss). Coagulated fibers (bottom) adjacent to normal fiber. × 600.

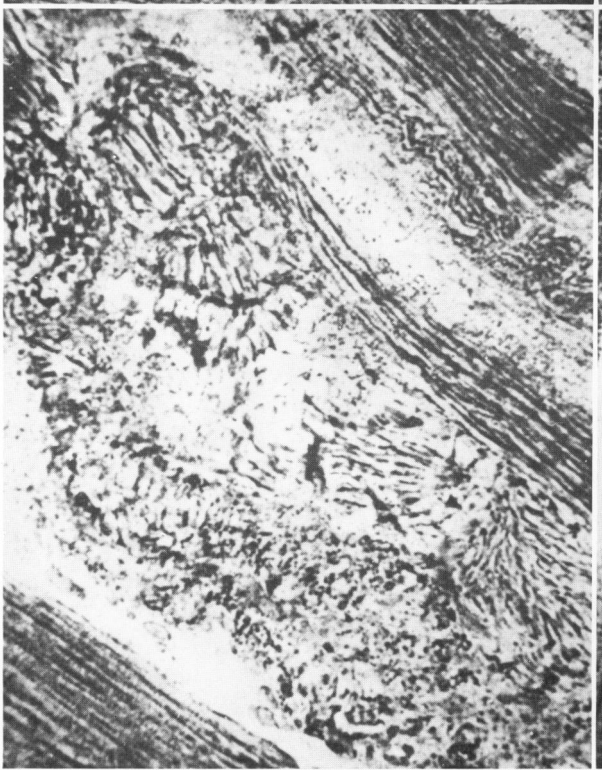


1A 1B



2A

2B



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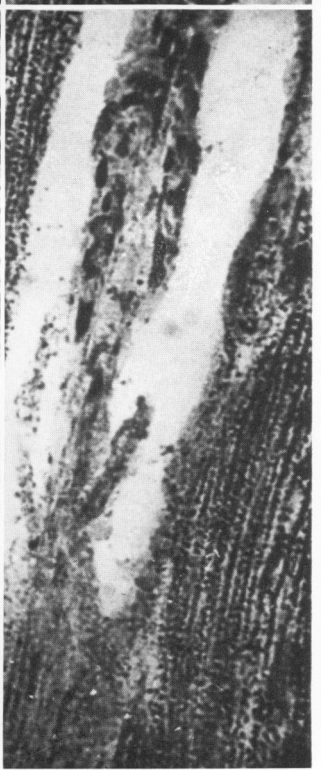

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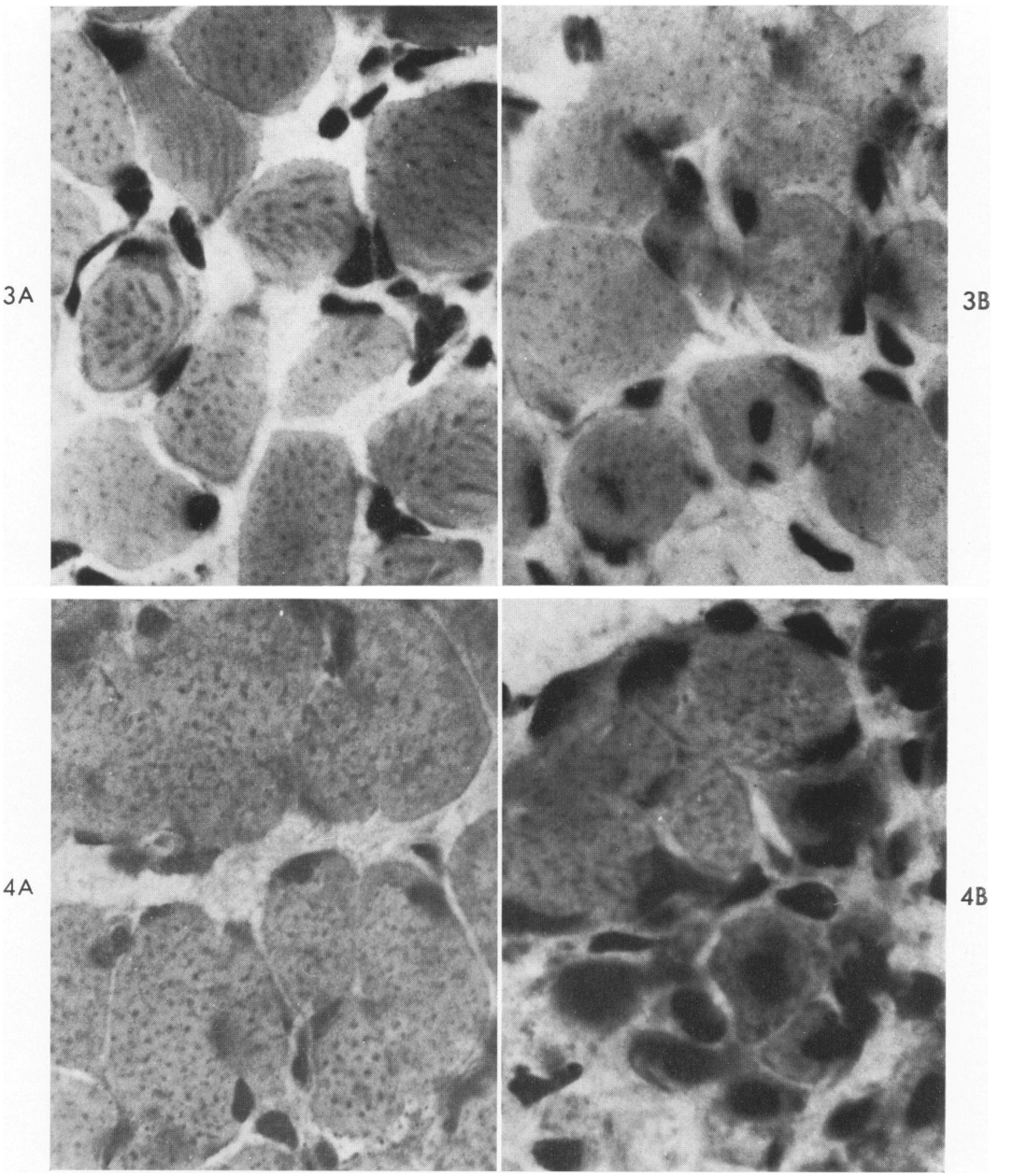

FIG. 2. Day 6. NADH-diaphorase: high activity (A); low activity (B). Early dystrophic changes: A, fiber contractions; B–D, clumpy accumulations of activity. × 600.

FIG. 3. Day 6. α-GPDH comparable in both normal (A) and dystrophic (B) genotypes. Dystrophic changes evidenced by centrally located nucleus. × 900.

FIG. 4. Day 1. GAPDH-staining reaction comparable in both normal (A) and dystrophic (B) genotypes. Activity occurs even in fiber with centrally located nucleus. × 900.

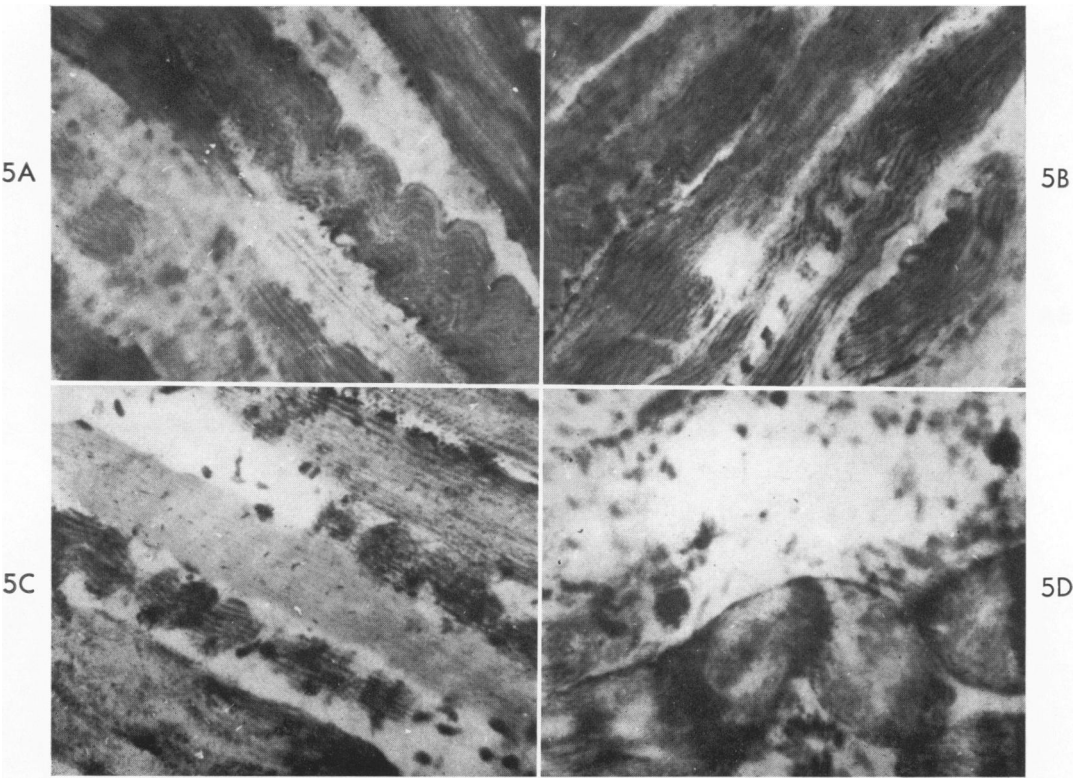

FIG. 5. Day 14. NADH-diaphorase. Dystrophic changes—only in certain fibers of bundle, fiber segments: A, contractions; A and B, enzyme-free spots; B, nuclear rowing (activity between nuclei located centrally in rows); C, segmental staining; D, longitudinal ridges. × 600.