THE HISTOPATHOLOGY OF PROGRESSIVE MUSCULAR DYSTROPHY AS REVEALED BY ULTRAVIOLET PHOTOMICROGRAPHY

By CHARLES L. HOAGLAND, M.D.,

ROBERT E. SHANK, M.D.,*

Lieutenant, Medical Corps, United States Naval Reserve

AND GEORGE I. LAVIN, PH.D.

(*From the Hospital and the Laboratories of The Rockefeller Institute for Medical Research*)

PLATES 1 AND 2

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Voluntary muscle constitutes approximately 40 per cent of the total weight of the body and is affected by a variety of diseases in which the primary process is associated with degeneration and atrophy of muscle fiber. Degenerative changes may also occur in a large group of secondary diseases of muscle. The latter diseases usually arise, however, as a result of pathological changes in the central or peripheral nervous system, and may, therefore, be differentiated sharply from those in the first group in which no neurological component is recognized.

Morphological studies of diseased muscle have yielded little or no information concerning the fundamental defect responsible for the extensive atrophy and dystrophy observed in the primary muscle disorders. Histopathological studies of the muscle in these syndromes have been, for the most part, cursory or superficial, and in no instance were the possibilities of modern cytological techniques fully explored. The recent development of a simplified quartz microscope, with the 2537 Å line of mercury as the light source, has made it possible to obtain ultraviolet photomicrographs of tissues fixed, embedded, and sectioned by routine methods (1, 2). Photomicrographs of tissue made by this technique show selective absorption of varying intensity, and reveal more detail than those obtained with visible light and ordinary staining methods. The technique has been shown to have particular advantages in the cytological study of muscle when it is desired to correlate certain morphological and physiological changes (2–4). In ordinary light the appearance of unstained sections of muscle is often misleading, since the image which is obtained under these conditions is due to inhomogeneity of the tissue and not to the presence or absence of material with selective absorption (5).

* Member of the Naval Unit at the Hospital of The Rockefeller Institute for Medical Research. The Bureau of Medicine and Surgery does not necessarily endorse the views or opinions expressed in this paper.

Marked differences in the absorptive capacity of the organic components of tissue may be expected to result when photographed with the 2537 Å line of mercury. The proteins have a maximum absorption at 2800 Å and the nucleo-proteins in the region 2600 to 2700 Å. Nucleic acid has a maximum absorption at 2600 Å, with an extinction coefficient about 30 to 60 times that of the proteins (8). Changes in tissue structure which result from differences in distribution or concentration should be readily detected by this method. Results, therefore, which are quite different from those obtained with conventional staining techniques may be expected, since the ultraviolet photomicrographs are a reflection principally of the chemical nature of the material, while photomicrographs of stained sections reflect merely the absorptive capacity of the dyes used in staining. It should be pointed out, moreover, that since the resolution obtained with ultraviolet light is approximately twice that obtained with visible light, a very considerable magnification of the photographs is possible.

Perhaps the best early accounts of progressive muscular dystrophy are contained in the publications of Duchenne (9), Gowers (10), Erb (11), and Landouzy and Déjérine (12). The disease is characterized by weakness and primary degeneration of certain groups of voluntary muscles, notably those of the pelvis, shoulder girdle, and lower extremities. Many clinical variations have been recognized, based on the age of onset and the location of affected muscles, but so far as is known at present, there are no essential pathological differences among the various types. The disease is familial and develops in its clearest manifestation during the period of transition from infancy to childhood. The pseudohypertrophic form appears early, and in the majority of cases is well advanced before the sixth year of life. Other forms are frequently delayed and may not appear until long after puberty.

During the past two years over 40 cases of progressive muscular dystrophy have been studied in The Rockefeller Institute Hospital. An account of the clinical studies, together with certain preliminary investigations on the metabolism in this disease, has been prepared and will appear elsewhere. The present communication deals with a histopathological study of material obtained from 15 biopsies of muscle.

Materials and Methods

Köhler, in 1904 (6), described a microscope of which the optical parts were made of fused quartz, thus providing a technique by which photomicrographs could be made by ultraviolet light. Since the time of Köhler a number of investigations have been made by means of this or similar instruments, including a study of normal frog muscle by Meigs in 1908 (7). The significance of the technique was immediately appreciated but its application has been limited, heretofore, by the difficulty of obtaining a sharp focus of the image. With the instrument described by Köhler (6) a high tension metallic spark was used as a light source and the various wave lengths of light were isolated by means of a quartz monochrometer. A focus was achieved first with visible light and an approximate focus in the ultraviolet was then obtained by setting the fine adjustment screw of the calibration apparatus in the same position as that used for the focus in visible light. A series of photographs were then made at various settings of the fine adjustment screw with the hope that one photograph might be in focus. An attempt was also made to calibrate the fine adjustment screw, but with only moderate success. In the apparatus devised by Lavin (1, 2), a much more simplified arrange-

ment has been effected. The light source is a mercury resonance lamp emitting 85 per cent of its energy in the form of the 2537 Å line. The component of energy in the range of visible light is removed by means of a liquid filter composed of cobalt and nickel sulfates. The microscope is focused directly by projecting the image of the tissue section on a fluorescent screen made of willemite. In this way the need for a complicated indirect focusing device is entirely obviated. The screen is placed in the position usually occupied by a photographic plate, and when a focus has been obtained the screen is removed and a photomicrograph made on a plate in the usual manner. Eastman metallographic plates were used.

The biopsies were performed on 10 cases selected to show the disease in its principal forms over the range of incipient to advanced stages. In some cases the biopsies were performed by ordinary surgical technique, following infiltration of the skin overlying the muscles with 5 per cent novocain. At times, when less tissue was required, biopsies were performed by means of a specially designed myotome (13). In 2 cases autopsies supplied multiple sections from many areas of voluntary muscle not available in biopsy technique. Tissue obtained at autopsy was placed in fixing solution within 6 hours after death. Biopsy material, on the other hand, was fixed in 10 per cent formalin immediately following its removal. The specimens were allowed to remain in the fixative for 24 hours and were then washed for 24 hours in several changes of distilled water at room temperature. The tissues were next carried consecutively through dehydrating mixtures of 50, 75, and 95 per cent alcohol, absolute alcohol, and finally xylol. The specimens were taken from xylol, embedded in paraffin, and cut on an ordinary microtome in sections of 5 μ thickness. For study with the quartz microscope the tissue sections were attached to a quartz slide with a trace of albumin. The paraffin was removed with xylol, which was in turn removed with absolute alcohol. While still moist the tissue was covered with glycerol and a quartz cover slip attached. Two or more photomicrographs were made from each tissue section after careful search had revealed the most satisfactory areas. In some cases sections of tissue were cut from each block at intervals of 500 μ, studied by means of visual examination on the fluorescent screen, and additional photomicrographs made of selected sections.

Description of the histological and pathological changes revealed by ultraviolet is exceedingly difficult because of the inappropriateness of the classical nomenclature for use in describing structures photographed in the ultraviolet. Certain analogies can be drawn, however, between structures photographed in ultraviolet light, and those structures which are seen in visible light as a result of the application of tissue stains, solely on the basis of size and location of the structures within a given cell. In this respect muscle tissue has proved to be a happy choice because of its highly specialized architecture. In tissues in which there is less adherence to a rigid form, however, the task of making a satisfactory comparison between structures revealed in ultraviolet light and those which are observed in visible light may be more difficult. It is evident that if ultraviolet microscopy is to be developed fully it will be necessary to revise and redefine the nomenclature of classical histology to the extent that it may take into account those structures which are due to selective absorption as well as those which are due to inhomogeneity of the tissue.

It is not the purpose of this communication to attempt a final definition of the various structures which are revealed by ultraviolet photomicrography in the muscles of patients with progressive muscular dystrophy. Attention can be drawn, however, to certain apparently specific changes based on broad differences in appearance of the tissue in the photomicrographs. Differentiation on the basis of finer structure must be postponed until we can distinguish with certainty between those changes which are due to pathological processes and those which are due to variation in fixation, dehydration, and sectioning of individual specimens of tissue. However, it is desired to point out that through increased resolution and clarity which have been achieved by photographing the unstained tissues in the ultraviolet light, the chances of revealing specific histological changes are much greater than is possible with stained tissues photographed in visible light. The necessity for an intensive study of various methods of fixation and sectioning, including studies of unfixed or lyophilized tissues, is clearly recognized before any final conclusions can be drawn regarding the specificity of the tissue structures revealed by ultraviolet photomicrography. A study of these problems is under way and the results will be recorded in future communications.

Description of Photomicrographs

Fig. 1 is a reproduction of an ultraviolet photomicrograph of an unstained longitudinal section of normal human pectoral muscle. Deeply absorbing transverse zones, spaced at regular intervals, and separated by alternate zones of low absorption, occur throughout the length of the muscle fibers. The over-all appearance of the photomicrographs is the familiar one of cross striation which has long been associated with voluntary muscle. It should be emphasized again, however, that structures revealed by visible light are due principally to changes in homogeneity of the specimen; or, in the case of structures seen in polarized light, to the phenomenon of micelle orientation, and that they may not, therefore, be compared directly with those structures which appear as a result of selective absorption in the ultraviolet region of the spectrum. Caspersson has concluded recently, however, that the isotropic zones, or dark striae, of muscle revealed by polarized light, do in fact correspond in position to the strongly absorbing areas seen in ultraviolet light (4). A comparison of the position of the striae appearing in polarized light with that occupied by striae appearing in ultraviolet light is also being made in this laboratory. The results will be reported later.

When the ultraviolet photomicrographs were subjected to further enlargement the strongly absorbing striae were revealed as lacking homogeneity, and appeared to be made up of a series of closely placed transverse lines (2). The fact that the transverse striae, which absorb deeply in the ultraviolet, are compound in nature is not altogether clear from the photomicrographs pub-

lished by Caspersson (4). It would appear, therefore, that the method devised by Lavin (1), in which focus is achieved directly by means of a fluorescent screen, has certain marked advantages over those techniques in which the focus is obtained first in visible light and only indirectly in the ultraviolet. When selected areas of the photomicrographs of muscle were enlarged to 8000 diameters or more, there was evidence of the presence of deeply absorbing longitudinal lines occurring at right angles to the transverse striae. The location of these columns in the ultraviolet photomicrographs was similar to the location of those columns seen by Richards, Anderson, and Hance in photographs of muscle made with the electron microscope (14). The relation of these areas of absorption in the ultraviolet to those which Caspersson has described as occurring in fatigued muscle is not clear. It is possible, however, that they may be identical.

Nuclei of the individual muscle cells absorb intensely in the ultraviolet. They lie at irregular intervals along the entire length of the muscle fibers immediately adjacent to the sarcolemma.

Fig. 2 is a reproduction of a photomicrograph of a transverse section of normal muscle made in the ultraviolet at a magnification of approximately 700. Punctate areas of intense absorption, corresponding in number and position to the individual fibrils of muscle are depicted clearly. The sarcolemma, which under ordinary conditions of staining and examination is difficult to demonstrate, was outlined clearly in the ultraviolet, and revealed considerable structure in the enlarged photomicrographs. In transverse sections of normal muscle the individual muscle cells appeared as sharply defined polygonal areas of very similar size and shape. This appearance is characteristic of normal muscle cells cut transversely, and may be regarded as due to the close packing of the muscle fibers in voluntary muscle. In these sections there was little evidence of areolar and fibrous connective tissue between the individual muscle fibrils. The polygonal form of the muscle cells and the scanty connective and areolar tissue separating them was in marked contrast to the picture presented by sections of diseased muscle.

Fig. 3 is a reproduction of an ultraviolet photomicrograph of a muscle section, removed by biopsy, from the vastus medialis of a subject showing moderately advanced progressive muscular dystrophy. Superficial examination showed that portions of the muscle fibers had been replaced by fat and fibrous connective tissue. The main outline of the remaining fibers was preserved. There were grotesque irregularities in the course of the fibrils which resulted in giving a "club-shaped" appearance to those fibers which were bent into acute angles. The endomysium was friable and unlike normal muscle had suffered considerable fragmentation through cutting with the microtome. There was marked variation in the appearance of the sarcolemma, with areas which showed intense absorption and others in which the absorption was so slight as to indicate that complete interruption in continuity had occurred.

The transverse zones showing selective absorption were thin and absorbed with less intensity than the broad, compound bands which were observed in transverse sections of normal muscle. In some areas the sarcolemma had an "eroded" appearance, showing partial or complete replacement by fibrous connective tissue. This is particularly well brought out in Fig. 9. The friable quality of the endomysium is also brought out clearly in this reproduction. Degenerative changes in muscle fibers were portrayed more clearly in photomicrographs of longitudinal sections of muscle than in those made from cross sections. Areas of dense absorption in cross sections of normal muscle were, as previously stated, punctate in character and arranged in a regular manner more or less equidistant from each other. In sections from dystrophic muscle these areas were coalescent and showed marked disorganization (Fig. 4). The relation of the areas of absorbing material in the muscle cross sections to the position of the "Cohnheim areas" of classical muscle histology, was not clear.

A series of photomicrographs from sections of muscle depicting various stages of involvement in progressive muscular dystrophy are reproduced in Figs. 5 to 8. The reproduction shown in Fig. 5 was made from a section of muscle which showed no detectable functional involvement, although the disease in certain other muscle groups in the subject was moderately advanced. It is clear, however, that retrogressive changes *were* present in this muscle as revealed by the appearance of scattered muscle fibrils undergoing marked degenerative changes. Only a few such fibers were found in this particular section and while clearly depicted in the ultraviolet photomicrographs they were not seen, after careful search, in alternate sections stained with hematoxylin and eosin and examined in visible light. It follows, that in subjects with progressive muscular dystrophy, moderately advanced changes may occur in certain muscle groups before functional alterations are detectable. Moreover, the assumption which is frequently made that normal muscle groups may occur in close relation to other groups which show definite dystrophic changes may be unwarranted. A study of serial biopsies by means of ultraviolet light, from functionally unimpaired muscles, as well as from those which show unmistakable changes, is needed before this point can be clearly established. Fig. 6 is a reproduction of a photomicrograph from muscle in which moderately advanced dystrophy was evident. Fibrous connective tissue in this muscle was just beginning to replace the degenerated fibrils. A study of a number of sections of muscle in the "pre-fibrotic" stage revealed that, although a given section of muscle may have shown intense absorption in the ultraviolet light, the absorbing areas were no longer well defined, and that areas of degeneration and fatty infiltration appeared in many of the fibers. At a later stage, *i.e.* that just preceding invasion by fibrous connective tissue (Fig. 7), the fibrils often showed marked loss in their capacity to absorb at 2537 Å. When this stage was reached the sarcolemma was no longer well defined, and much of the inner

architecture of the fibrils appeared to have been lost. In each photomicrograph the exposure time was held constant, and since the sections were cut at the same thickness, differences in intensity of absorption, as registered in the ultraviolet light by the various sections, were comparable. Reproduced in Fig. 8 is a photomicrograph of muscle showing the final stages of change associated with progressive muscular dystrophy. Replacement of muscle parenchyma by fibrous connective tissue was nearly complete. Even in this instance, however, careful inspection revealed an occasional residual muscle fiber. Alternate sections of this tissue, stained with hematoxylin and eosin and examined in visible light, revealed no structure which could be identified with certainty as muscle fiber. In the ultraviolet, on the other hand, enough of the architectural form of the fibers remained to make their identification certain. It would appear, therefore, that residual fibers in various stages of degeneration may be seen in areas when the tissues stained by ordinary histological methods reveal nothing but fat and fibrous connective tissue.

Fig. 9 is a reproduction of a photomicrograph of a transverse section of muscle and was described briefly in connection with Figs. 1 to 3. The changes revealed in this photomicrograph presented an interesting contrast with those seen in the photomicrographs reproduced in Fig. 10. The latter photomicrograph was made from a section of adductor muscle removed at autopsy from a case of advanced amyotonia congenita. In the photomicrograph from the section of dystrophic muscle (Fig. 9) the areas of degeneration appeared to begin and end abruptly at the transverse striae. Although a given zone of degeneration may have covered a variable number of striae it was sharply divided from surrounding areas of normal muscle. In the sections removed from the muscles of the case with amyotonia congenita, on the other hand, the degenerative changes were associated with longitudinal splitting of the muscle fibers, and the fat was almost wholly confined to the fibrillar interstices. No evidence of loss or diminution in absorption of striae was recognized. Unlike the changes seen in the muscles in progressive muscular dystrophy, the muscles in amyotonia revealed no alteration other than that associated with the interfibrillar deposition of fat. Additional histological study of the muscles in amyotonia congenita with the ultraviolet technique is needed before any definite conclusions can be drawn regarding the nature of the specific muscular lesions existing in this disease.

DISCUSSION

The fact that ultraviolet light photomicrography has certain definite advantages over ordinary photomicrography in visible light, as a technique for the demonstration of tissue structure, has been known since 1904. Indeed, in the hands of Caspersson (4), Meigs, and others (7), the technique has been applied with some degree of success, not only for the purposes of providing greater

resolution and clarity in photomicrographs, but in an attempt to demonstrate the nature of structural material in tissues as well (15, 16). The fact that the image produced by ultraviolet light is dependent to a marked extent on selective absorption of tissue components makes it possible to obtain photomicrographs with greater resolution and better definition than can be obtained in visible light.

The histopathological changes associated with muscle disease were chosen as a problem for the extension of the ultraviolet technique for two reasons: First, because of the authors' interest in diseases of muscle; and secondly, because the specialized structure of muscle is such that it allows a fairly satisfactory comparison to be made between the larger structures revealed in ultraviolet photomicrographs and those which are rendered visible by staining and photography in ordinary light.

Reproductions of photomicrographs made by the ultraviolet technique which depict a variety of histological changes associated with various stages in the development of progressive muscular dystrophy, have been selected to accompany this report. No attempt has been made to describe minutely all the microscopic changes which have been revealed by the ultraviolet technique to occur in the muscles in progressive muscular dystrophy. Much additional study of muscle tissue, both normal and diseased, and under other conditions of fixing and sectioning, will be necessary before the specificity of the lesions can be fully defined. Studies are being made on fixed and unfixed tissues in the ultraviolet, and on tissues prepared by the method of lyophilization and anaerobic imbedding as described by Packer and Scott (17).

Ultraviolet photomicrographs of unfixed surviving muscle of insects have been obtained recently by Caspersson (15). With a more exact method of focusing it should be possible to obtain additional detail and sharper definition than that shown in Caspersson's reproductions. Because of the difficulty in securing sections of unfixed mammalian muscle, in sections sufficiently thin for microscopic examination, the problem of obtaining photomicrographs of this tissue is considerably more difficult than for insect muscle. It is hoped, however, that this difficulty can be overcome, and studies to this end are under way.

CONCLUSIONS

Results have been presented of the application of a simplified technique of ultraviolet photomicrography to a study of the specific lesions in muscle in subjects with progressive muscular dystrophy. An exact description of the histological changes occurring in this syndrome, as revealed by photomicrographs in ultraviolet light, is difficult at this time because of the lack of an adequate system of nomenclature. Attention has been drawn, however, to lesions of consistent character, found in sections of muscle removed at biopsy, which appear to be specific for the disease.

The method of simplified ultraviolet photomicrography possesses certain marked advantages over those classical methods of histology and pathology which depend on staining and examination of specimens in visible light. Not only is greater resolution achieved with ultraviolet light photomicrography but the image which is obtained in the ultraviolet may provide some idea of the chemical nature of the tissue as well, since it results chiefly from the selective absorption of light by proteins, nucleoproteins, and nucleic acids.

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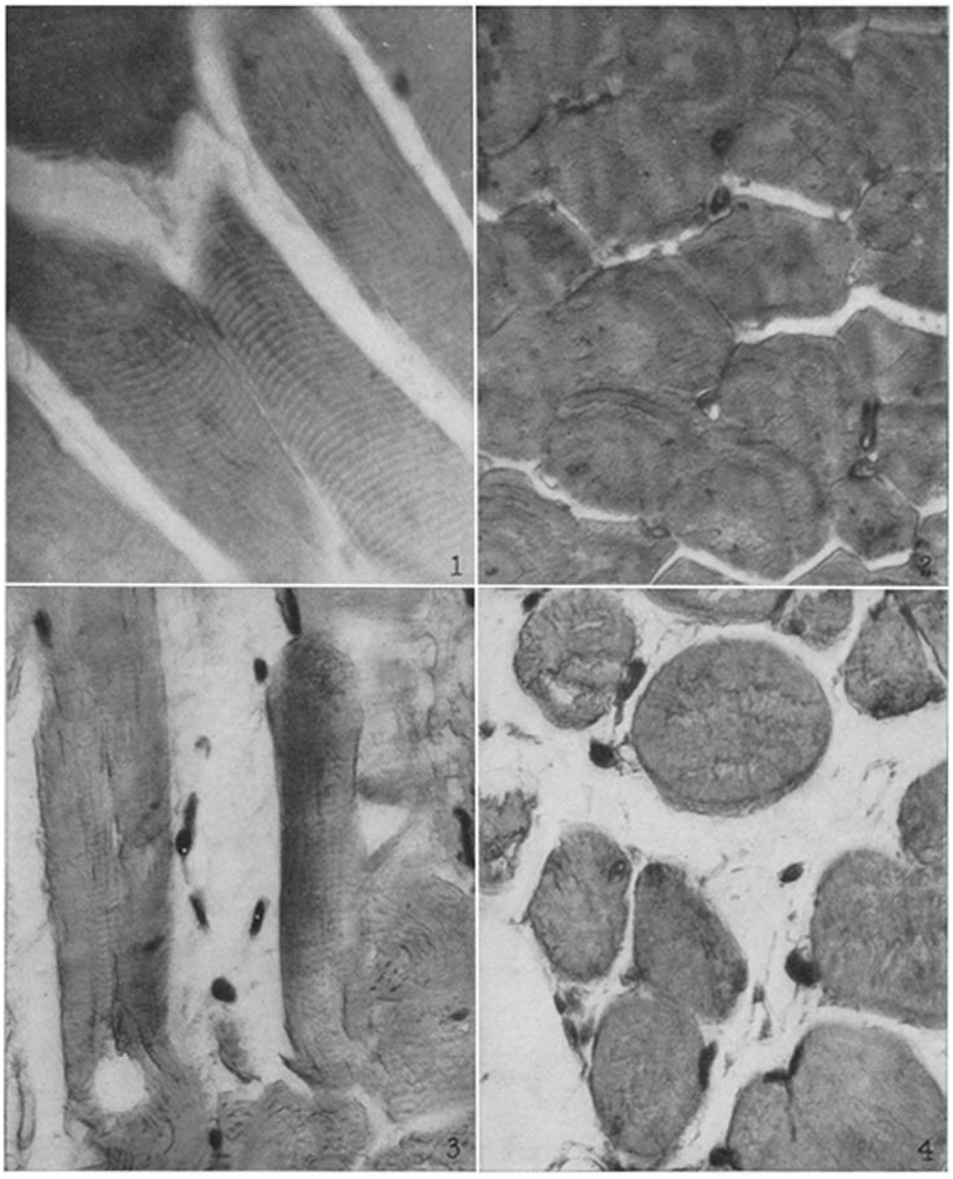
EXPLANATION OF PLATES

5 μ sections of voluntary muscle, fixed in formalin, embedded in paraffin, and photographed in the ultraviolet at 2537 Å.

PLATE 1

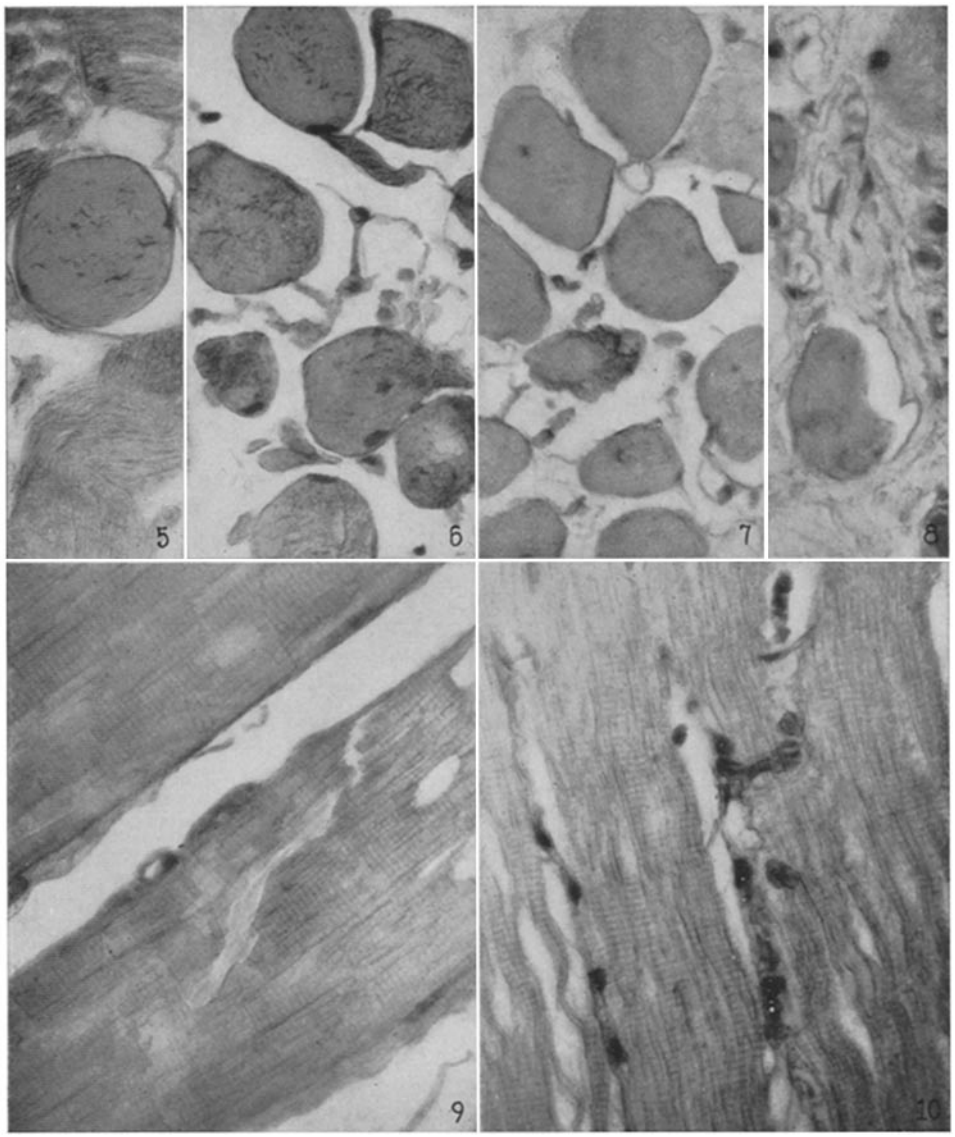
FIGS. 1 to 4. Ultraviolet photomicrographs of longitudinal and transverse sections of human muscle. Figs. 1 and 2, normal subject; Figs. 3 and 4, patient with moderately advanced progressive muscular dystrophy. ×700.



(Hoagland *et al.*: Histopathology of progressive muscular dystrophy)

PLATE 2

FIGS. 5 to 10. Ultraviolet photomicrographs of human muscle. Stages in the degeneration of muscle in progressive muscular dystrophy. Fig. 5, early, Fig. 6, moderately advanced, Fig. 7, advanced, and Fig. 8, final stage. Comparison between changes seen in a longitudinal section of muscle showing moderately advanced dystrophy (Fig. 9) and in degeneration of muscle in amyotonia congenita (Fig. 10). ×700.



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