AN ATLAS OF MUSCLE PATHOLOGY IN NEUROMUSCULAR DISEASES

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PREFACE

THIS Atlas is the result of a study by four independent investigators of 121 cases of muscle disease which were biopsied in an attempt to define the histological changes. It is thus a part of a co-ordinated study of neuromuscular diseases being conducted at the National Institute of Neurological Diseases and Blindness. Eleven additional cases have been obtained from the Armed Forces Institute of Pathology and one from Cornell University Medical College.

One of the major difficulties, when this investigation began, was the lack of precise definitions of fundamental histological alterations in muscle. Such commonly used words as *necrosis*, *degeneration* and *inflammation*, for example, were subject to a variety of interpretations both in the literature and among the present investigators. Furthermore, no mutually acceptable example could be found under the microscope. In part this difficulty seemed to be due to an inability momentarily to forget the clinical diagnosis and the consequent implications concerning the pathogenesis of the diseased state. In this day when laboratory investigators find clinical information essential before giving an opinion, this difficulty may appear naïve, but we have repeatedly demonstrated to ourselves how knowledge of the clinical diagnosis may influence our description as well as our interpretation of the histological sections.

An experiment was therefore devised to attempt to answer two questions: (1) What are the fundamental reactions of muscle to disease? (2) With what clinical entities are these reactions associated? If these two questions could be satisfactorily answered, the value of muscle biopsy in the differential diagnosis of neuromuscular diseases would be clear, and further research might be planned on a firmer foundation.

Accordingly, a tentative scheme for analysing and recording abnormalities of muscle was devised, avoiding as nearly as possible any term which was unduly suggestive of more than could be seen microscopically. Muscle biopsies identified only by the accession number in the Section of Clinical Neuropathology were then examined and coded by each of us individually, subsequently reviewed by the group, and a consensus reached and coded. Only then was reference made to clinical observations and diagnostic impressions. After final clinico-pathological correlation of the cases, it was felt that the definitions of histological changes which we had accepted and the method of analysis which we had used allowed us to give a reasonably satisfactory answer to our initial two questions. These might be paraphrased by asking: How much can the pathologist with ordinary techniques contribute in the differential diagnosis of neuromuscular diseases?

As in the experiment described above, the Atlas is divided in two parts. In Part I the various types of histological reaction of muscle to disease are defined and illustrated without regard to the clinical impression. Attempts have been made to choose illustrations which are not ambiguous and which

accurately portray the varieties of change which we have seen. Where possible, black and white reproductions have been used; however, in cases where slight differences in shade or colour are important, illustrations in colour are necessary.

In Part II the significance of these reactions is determined by the correlation of various combinations with the clinical observations. Both positive and negative correlations are made, and both aspects emphasized in the histopathological characterization of the various clinical entities. Again, difference in current nomenclature presented difficulties in interpretation, particularly where clinical diagnoses such as *myositis* and *muscular dystrophy*, for example, imply a histopathological definition. We therefore attempted to define in simple terms the various clinical syndromes we encountered without reference to the histological features.

This Atlas must in no way be considered as an attempt to define all of the changes which may occur in muscle or all of the diseases to which muscle is susceptible. It must be kept in mind that the patients were referred to us with the diagnosis of one of the neuromuscular diseases, such as *muscular dystrophy*, *myositis*, myasthenia gravis, myotonic syndromes, neurogenic muscular atrophies and *flabby infants*. It is hoped, however, that this attempt to define some of the common reactions and diseases will be helpful to other investigators in the field of neuromuscular disease.

THE AUTHORS.

February 1957.

ACKNOWLEDGMENTS

We wish to express our gratitude to the patients and to their referring physicians who have made this study possible. We also wish to thank our clinical associates, Doctors Gunter R. Haase, Israeli A. Jaffe, Marvin C. Korengold, Kenneth R. Magee, Lewis P. Rowland, Glen A. Drager and Charles E. Wells, who have directly cared for many of the patients and who have performed many of the biopsies. Most of the histological sections were prepared by Mr Daniel A. Williams, and all of the photographs were taken by Mr Fred H. Meiller, to both of whom our thanks are due. Case 533 was obtained through the courtesy of Doctor John G. Kidd, Department of Pathology, Cornell University Medical College. Cases 905-914 and 965 were obtained from the Armed Forces Institute of Pathology. We wish to thank the director, Brig.-Gen. Elbert DeCoursey, for the opportunity to study these cases. To Dr Albert J. Dalton of the National Cancer Institute we are indebted for the electron microscopic preparation (Fig. **1**, 4). We are also indebted to the Muscular Dystrophy Associations of America, Inc., for their financial support towards the preparation of the colour prints.

THE AUTHORS.

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PART I

HISTOPATHOLOGICAL REACTIONS OF MUSCLE

PART II

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CHAPTER 1

STRUCTURAL CHANGES IN THE MUSCLE FIBRE: LOSS OF CROSS-STRIATION, CLOUDY AND GRANULAR CHANGES, FLOCCULAR CHANGES [1] AND PHAGOCYTOSIS

NORMAL human muscles are composed of muscle fibres surrounding which is a variety of interstitial tissues (*cf.* Chapters 6 to 8). The muscle fibres are long, cylindrical, multinucleated cells generally considered to be covered by a thin, membranous structure, the *sarcolemma*. The actual structure of the sarcolemma remains in doubt, and can only be differentiated from the surrounding reticulin of the *endomysium* by electron-microscopy or by special stains, such as Lillie's allochrome method (Lillie, 1952). With the allochrome technique, both sarcolemma and reticulin are first stained red by the periodic acid-Schiff (PAS) technique, but with the addition of picro-methyl-blue, the reticulin changes colour (*i.e.*, it is 'allochromic,' usually staining blue), whereas the sarcolemma remains red (Fig. **1,** 1). With shrinkage the sarcolemma may remain either with the reticulin or with the muscle fibre. Lillie believes that fine reticulin fibres insert into the outer part of the sarcolemma and that the sarcolemma is extracellular, but we have not been able to distinguish between the sarcolemma and the surface of the muscle cell. By electron-microscopy (Bennett, 1954), reticulin fibrils can be distinguished external to the sarcolemma, which consists of a double membrane forming the surface of the muscle cell.

The cytoplasm of the muscle fibre consists of sarcoplasm in which are embedded thin, cross-striated *myofibrils*. The myofibrils run parallel to one another in the long axis of the muscle fibre. They are composed of alternating bands, birefringent; anisotropic (*A or Q band*) and isotropic (*I or J band*) under polarized light. Under ordinary light the appearance is reversed, so that the A band appears dark and the I band appears light (Fig. **1,** 2). In the middle of I band is the Z disc. On either side of the Z disc, *N bands* are apparent by electron-microscopy within the I band. The segment between two Z discs is called a *sarcomere*. In mammalian muscle a sarcomere is generally about 2 micra long and comprises one complete A band and two half I bands on either side of the A band. The structure of the A band can be fully appreciated only in the relaxed condition, when the *M membrane* is apparent in the middle. With suitable magnification a narrow *H band* is seen on either side of the M membrane, and an *E band* next to the H. The width of the E band depends upon the degree of stretch. With the electron microscope (Fig. **1,** 3), myofibrils

[1] " Plasmodial regression " (Durante, 1902; Adams *et al.*, 1953), or " sarcoplasmic degeneration " (Brodal *et al.*, 1953).

are seen to be composed of still finer, long, thin *myofilaments* running continuously through the A and I bands. The thickness of these filaments is about 400 A°; they are considered to be the ultimate structures responsible for the contraction of the fibre.

The *sarcoplasm* of the muscle fibre fills the spaces between the myofibrils, but following fixation the spaces between the fibrils often appear clear. The sarcoplasm contains *sarcoplascine reticulin* and *interstitial granules* within a serous *matrix*. The sarcoplascine reticulin courses longitudinally and transversely through the muscle cell, being concentrated in the region of the Z discs and attaching to the sarcolemma (Bennett, 1955), (Fig. **1,** 4). Among the interstitial granules may be distinguished *sarcosomes* (Harman, 1955), mitochondria, lipochrome pigment, lipoid granules and glycogen. Sarcosomes occur at both the I and A discs, but their exact nature is not known. Glycogen and lipids occur in two forms: larger granules are scattered throughout the fibre, whereas very small granules are often concentrated in the I band.

Architectural changes as seen through the light microscope consist of the following: (1) *loss of cross-striations*, and (2) loss of myofibrils with the development of *cloudy*, *granular* and *floccular* appearances. After the *loss of their cross-striations*, myofibrils are still visible as longitudinal threads, but as an isolated event this is not common. Under ordinary light, cross-striations may be invisible, particularly with H & E or Van Gieson stains, when they are readily apparent under polarized light. One must be careful to orient the plane of polarization correctly for each fibre examined. Further alteration in the myofibrillary structure results in complete loss of longitudinal threads with the development of a hazy, *cloudy* appearance (Fig. **1,** 5). *Extreme contraction* of a muscle fibre may result in a relatively homogeneous appearance on cross-section (Fig. **1,** 6) which should not be confused with a cloudy change. On longitudinal section (Fig. **1,** 7) such fibres may have such short sarcomeres that they appear diffusely birefringent (Fig. **1,** 8). The structure of the muscle fibre may be converted into a mass of fine granules (*granular change:* Figs. **1,** 9–10), or of larger *floccular* collections of protoplasm (Fig. **1,** 11). Floccular changes are generally accompanied by the invasion of leucocytic elements, especially phagocytes (Figs. **1,** 12–13). Such flocculation may not be complete, however, and portions of myofibrils may be found in both cross and longitudinal sections, giving a vermicelli-like appearance (Figs. **1,** 14–15). The nature of the granular and floccular particles is not known.

Small fibres (*i.e.*, less than about 30 micra in diameter) often lose their cross-striations and myofibrils, and appear floccular, but phagocytosis of such fibres is unusual. On the other hand, fibres may occasionally be decreased in size to 5 micra or less and still maintain myofibrils with cross-striations (Fig. **1,** 16). Structural changes in small fibres may have a significance different from that of similar changes in larger fibres (Part II).

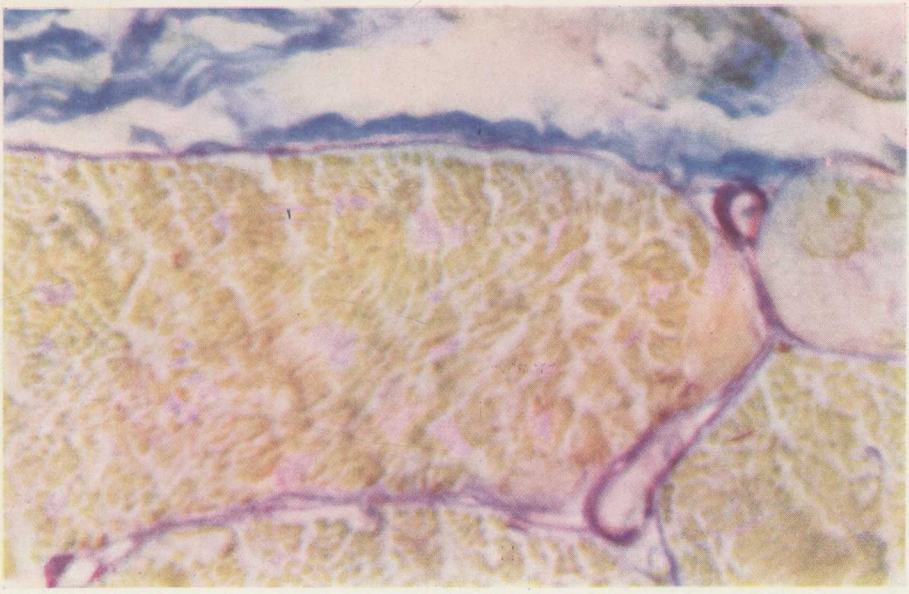

FIG. 1, 1

Cross-section of muscle fibres with the sarcolemma in red and the reticulin of the endomysium and perimysium in blue. The distinction is best seen where they are not sectioned obliquely. (8729-718-C, Bouin's, Lillie's allochrome, ×1800.)

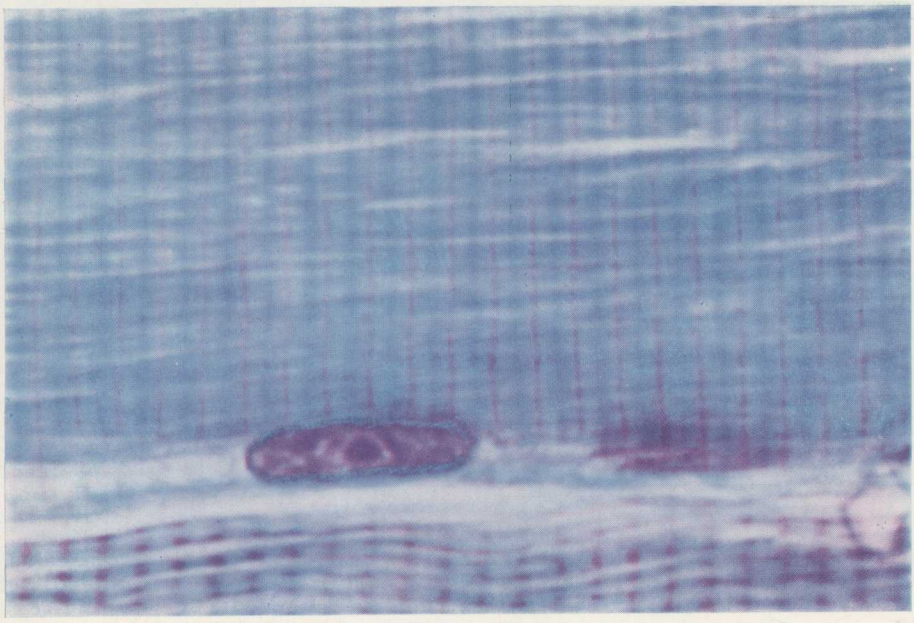

FIG. 1, 2

Longitudinal section of normal appearing muscle fibre. The Z discs are red and situated in the middle of the pale I band. The A band is dark. (8787-63-D, Bouin's, trichrome, ×2400.)

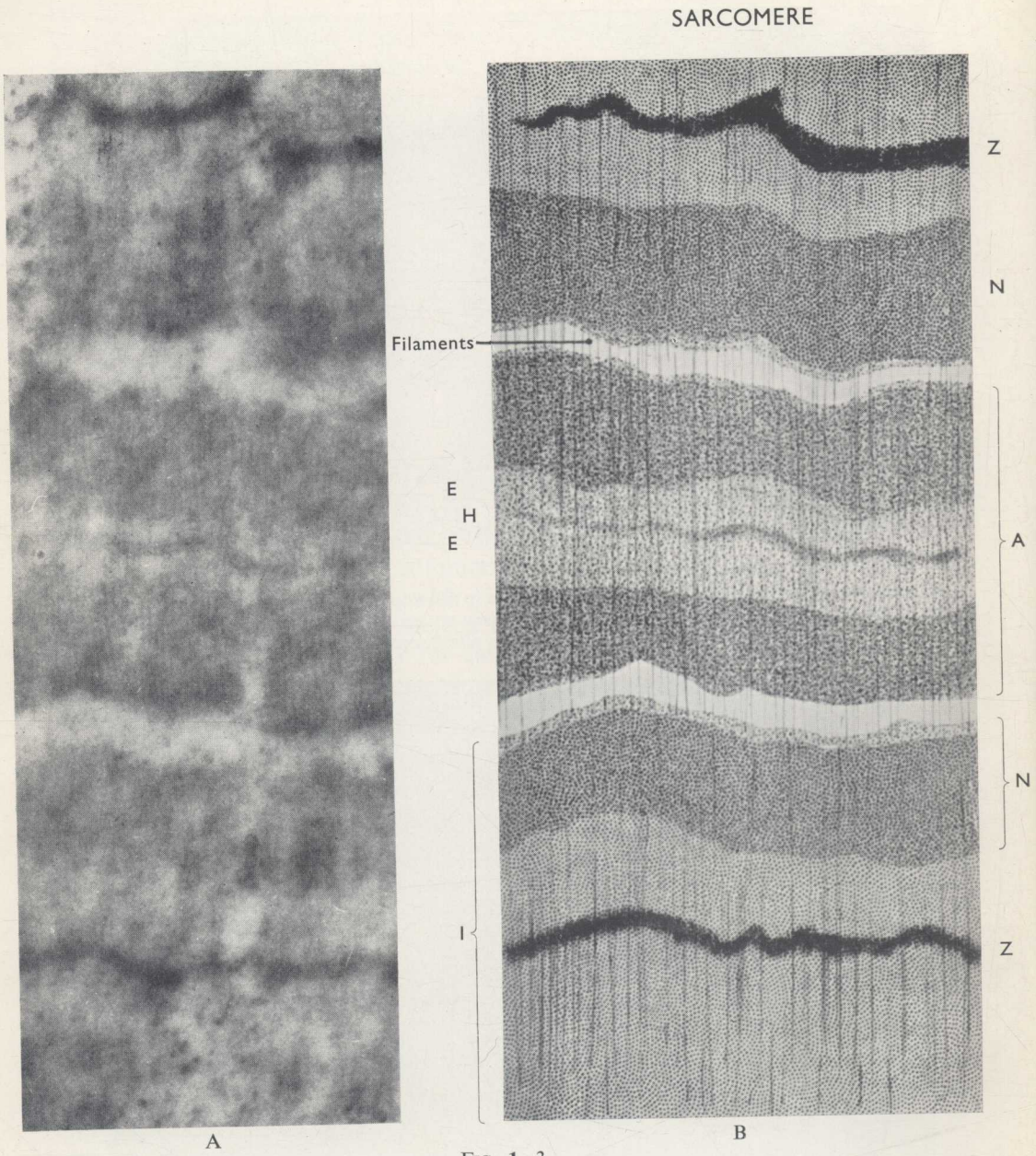

FIG. 1, 3

Electron micrograph of a sarcomere from human quadriceps taken at biopsy and fixed immediately in chrome-esmium (*p*H 7·2). Filaments of approximately 10 mμ in diameter and the characteristic discs and membranes of skeletal muscle are visible. The horizontal line near the bottom of the figure represents 1 μ. (Albert J. Dalton, National Cancer Institute.)

A, Electron microscopic preparation (×30,000).
B, Diagram showing position of bands.

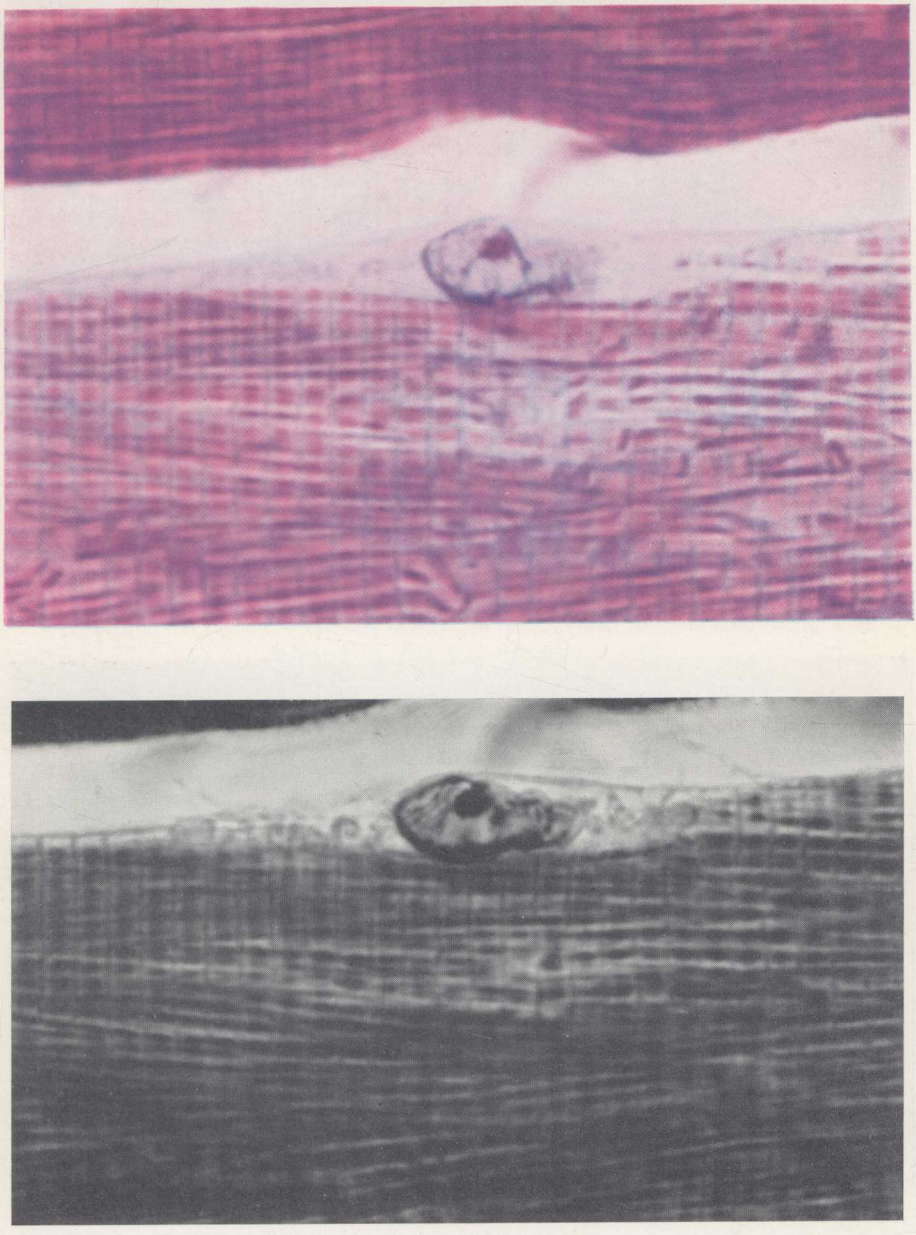

FIG. **1**, 4

Longitudinal section of muscle fibre in which the Z discs appear to be attached to the sarcolemma. Electron-microscopy (Bennett, 1955) reveals this "attachment" to consist of sarcoplasmic reticulin. (8808-862-A, Zenker's, trichrome, ×2200.)

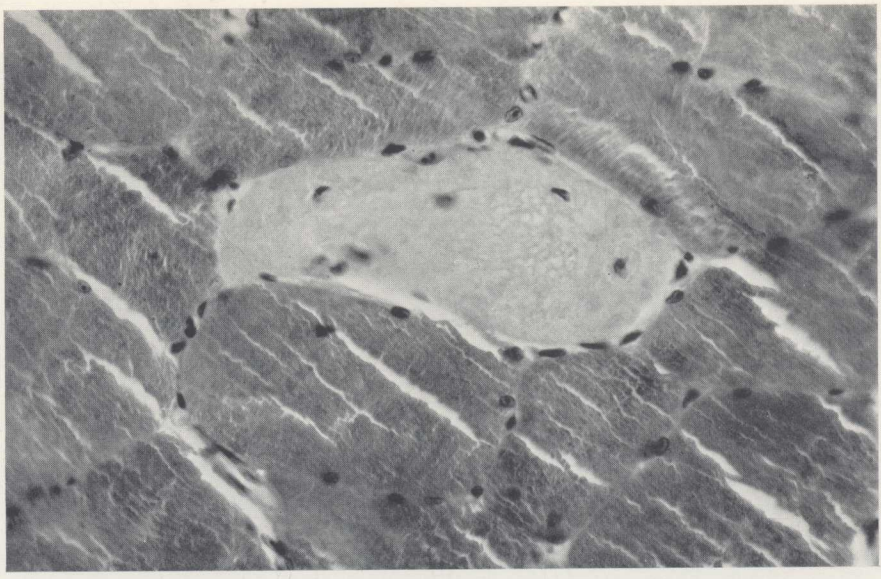

FIG. **1**, 5

Cloudy appearance of transversely sectioned muscle fibre. (See also Fig. **10**, 2.)
(8713-915-C, Bouin's, trichrome, ×330.)

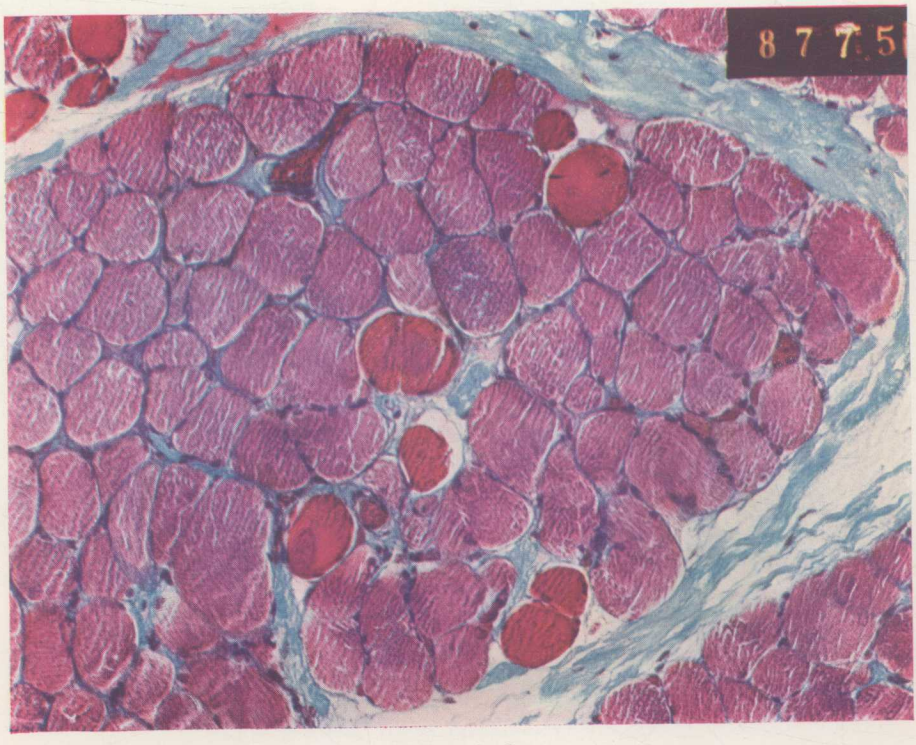

FIG. **1**, 6

Cross-section of muscle fibres, many of which are dark red and homogeneous due to extreme contraction. Compare with Figs. **1**, 7 and 8. (8775-117-A, Bouin's, trichrome, ×210.)

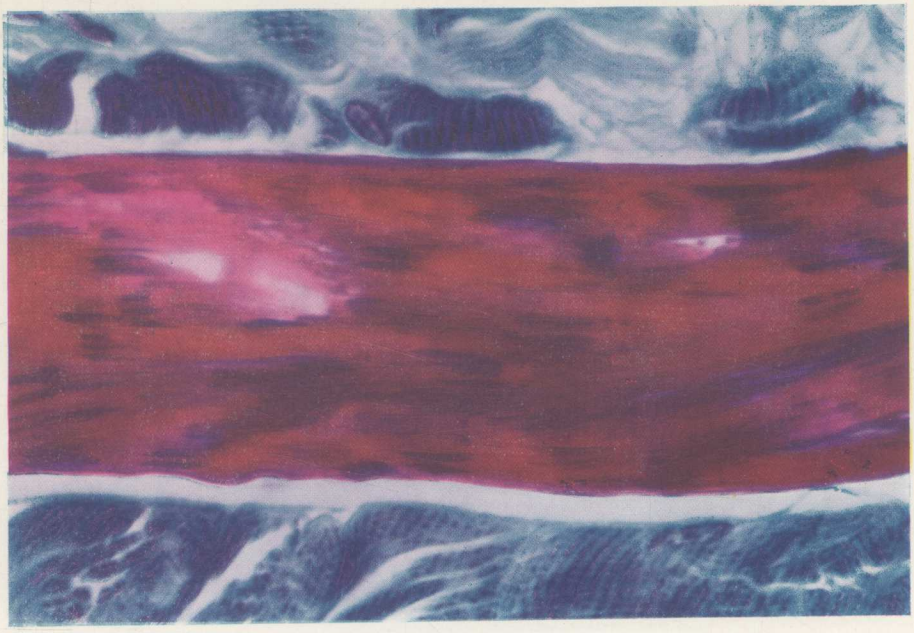

FIG. **1**, 7

Longitudinal section of severely contracted fibre, similar to those seen in Fig. **1**, 6. (8784-117-A, Bouin's, trichrome, ×1050.)

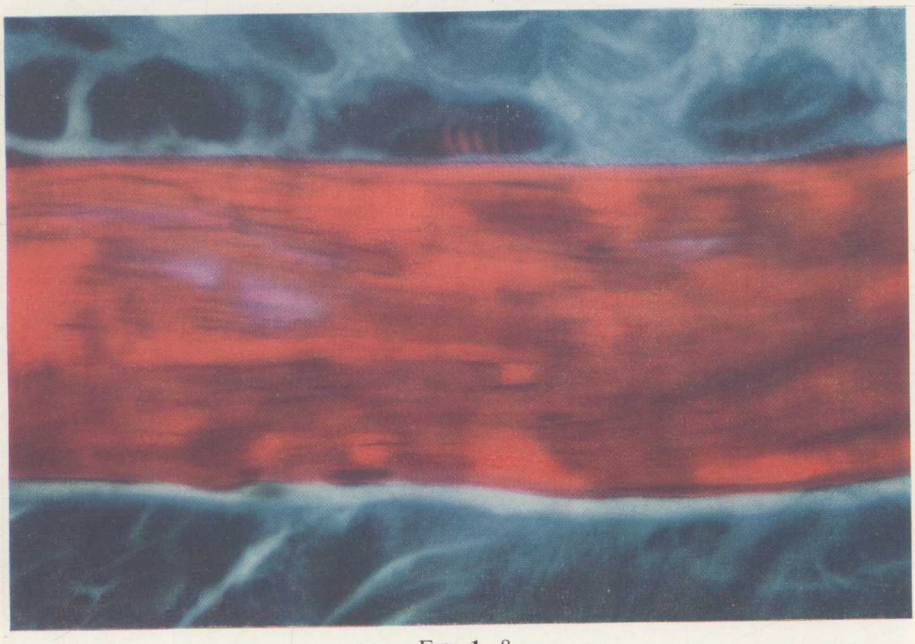

FIG. **1**, 8

Same fibre under polarized light. Note diffuse birefringence. Under still greater magnification very closely spaced cross-striations could be seen. (8781-117-A, Bouin's, trichrome, ×1050.)

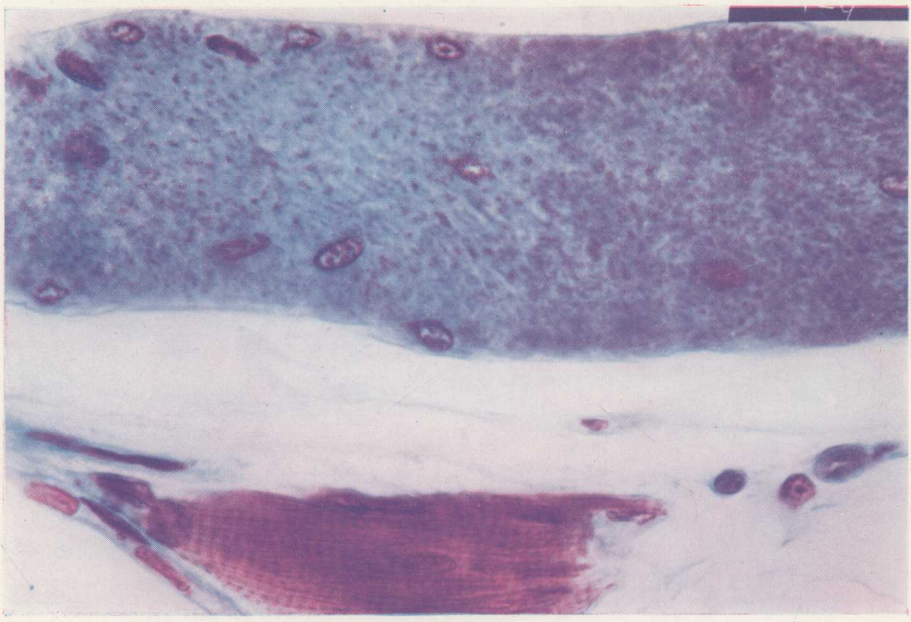

FIG. **1,** 9

Granular muscle fibre in longitudinal section. Small birefringent particles scattered throughout the fibre were visible with polarized light. (8647-39-A, acetic Zenker's, trichrome, ×800.)

FIG. **1,** 10

Granular fibre in cross-section. (8661-603-A, Bouin's, trichrome, ×1700.)

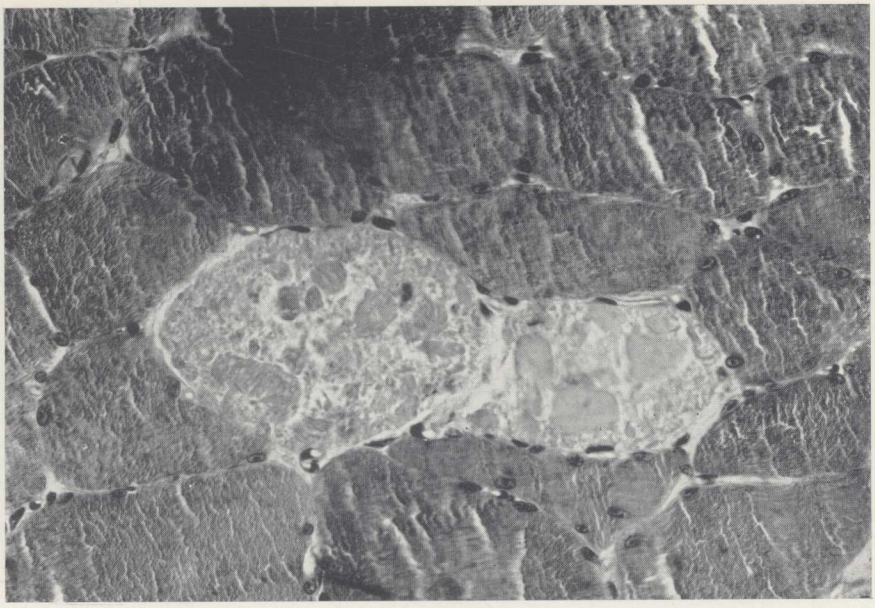

FIG. **1,** 11

Muscle fibres undergoing floccular change. (See also Fig. **10,** 2.)
(8712-915-C, Bouin's, trichrome, ×330.)

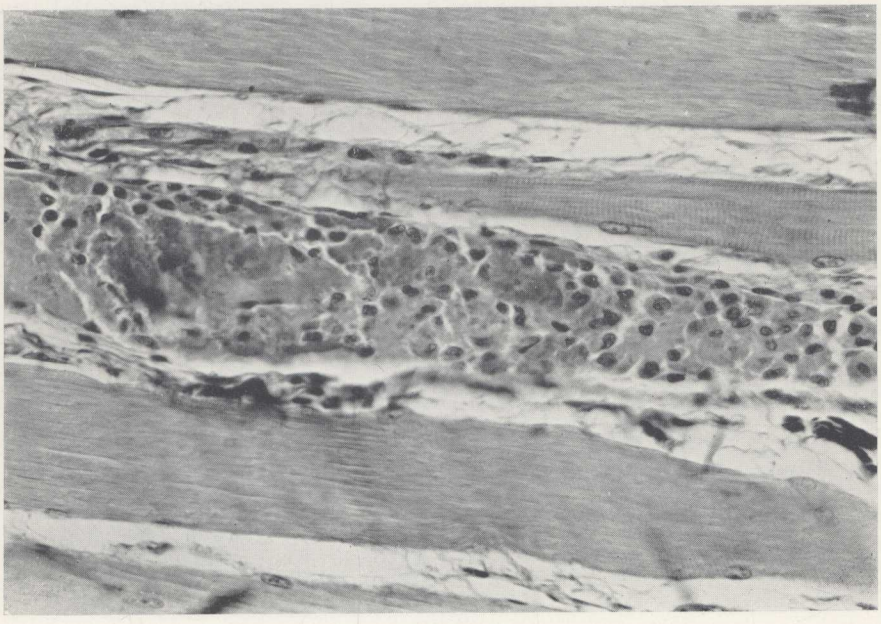

FIG. **1,** 12

Muscle fibre undergoing phagocytosis and floccular change.
(8636-60-A, acetic Zenker's, trichrome, ×330.)