STUDIES ON STRIPED MUSCLE STRUCTURE

IV. INTERCALATED DISCS IN VOLUNTARY STRIPED MUSCLE

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ONE FIGURE

INTRODUCTION

In a series of papers¹ on the intercalated discs of cardiac muscle, the hypothesis was developed that these structures represent modified irreversible contraction bands. As stated in an earlier article (5), if this interpretation of the intercalated discs of heart muscle is correct, then we should expect to find similar structures also in skeletal muscle, under certain conditions. Continued search had, however, until recently been only rewarded by negative results. While giving the course in Histology at the College of Physicians and Surgeons, Columbia University, during the summer session of 1918, I noticed in the sections of human voluntary striped muscle (sec. no. 294) included in the students' loan sets of slides, certain peculiar structures which seemed very suggestive of the intercalated discs characteristic of cardiac muscle. Dr. George S. Huntington has very kindly given me permission to use this material for study and description. The data now available only give the information that the specimen came from a leg removed at operation by Dr. W. C. Clarke, that the tissue was fixed in formalin, imbedded in celloidin, and stained with Mallory's phosphotungstic-acid hematoxylin. The preparations are superb.

The only reference to intercalated discs in skeletal muscle which I have been able to find appears in a monograph by

¹ See bibliography in article by Jordan and Banks. *Am. Jour. Anat.*, vol. 22, p. 285, 1917.

Dietrich (1), who mentions that H. B. Schmidt demonstrated at the meeting of the Pathological Society in Erlangen in 1910 preparations of voluntary striped muscle in which occurred bands which Schmidt regarded as having the same structure as the intercalated discs of heart muscle. Dietrich, however, after careful study of Schmidt's preparations, disputes the accuracy of such an interpretation. Dietrich's description of these structures as "irregular stripes which cross the muscle bundle in successive waves," and his identification of them with the abundant contraction waves frequently seen in the cardiac muscle of human cadavers (p. 11), make it quite certain that there is no close similarity between the structures in our specimen and those in Schmidt's material. I feel the more convinced of this conclusion since certain sections through the upper third of the cat's esophagus in my collection show bands which correspond exactly to those described by Dietrich in Schmidt's sections; these bands have nothing in common in the way of essential details with those in the sections of human leg muscle. In the latter material occur definite and regular discs related to the telophragmata in a manner identical with the intercalated discs of *Limulus* and vertebrate heart muscle; in the former material occur irregular contraction waves, spanning the entire width of the tunica muscularis in the case of the esophagus and involving a variable number of sarcomeres. The discs of the leg muscle are modified single contraction bands, which apparently failed or were unable to reverse in certain sharply limited locations; the bands of the esophageal muscle are widespread contraction waves fixed either in rigor mortis or by the action of the preserving fluid.

It would be of prime interest to know the morbid conditions which necessitated the removal of the limb from which our specimen was taken. Such information might indicate the specific factors operating in the production of intercalated discs. At present we can only surmise, in accordance with our general theory regarding the formation of such discs in heart muscle, that the special conditions under which discs originate include relatively violent or prolonged tensions or exceptional strains,

such as may accompany long-continued rhythmic contraction. The correlation of such discs with specific physiologic, pathologic, and experimental conditions would seem to offer a worthwhile field for future investigations.

In order to determine whether these peculiar structures in this specimen might possibly be a common characteristic of human leg muscle, I prepared sections from three other amputated legs which had been preserved in formalin. For two of these specimens I am indebted to Dr. W. C. Clarke, of Columbia University; for the third to Dr. Stephen H. Watts, of the University of Virginia. None of these specimens contained similar discs; only occasional areas of fibers were at a midphase of contraction; the sections indicated that these muscles were for the most part in a condition of repose. In order to test the further possibility that the discs of our specimen might be the result of the special staining technic, sections of these three specimens of leg muscle were also stained with the phosphotungstic-acid hematoxylin. These preparations again gave no evidence of similar discs. In addition, pieces of striped voluntary muscle of the frog were fixed in strong Flemming's solution, in the picro-acetic solution, and in 95 per cent alcohol, and stained respectively with iron-hematoxylin and phosphotungstic-acid hematoxylin. These six sets of slides showed essentially identical conditions: the Q-disc was bisected by an H-disc of considerable width, but nothing suggestive of intercalated discs was discernible.

It is required first to establish the homology between these discs in our specimen of leg muscle and those of cardiac muscle, vertebrate and *Limulus*. This is a relatively easy matter. The description of these discs will show a detailed similarity to cardiac intercalated discs amounting practically to a morphologic identity. While it is of much theoretical interest to have discovered genuine intercalated discs in skeletal muscle, and while the discovery adds support to our interpretation of these discs in cardiac muscle as modified irreversible contraction bands, it is clearly recognized that it does not prove that the theory is entirely correct. But a prediction made on the basis of this theory has now been fulfilled. The theory, however, involves

the whole question of the mechanism of muscular contraction, concerning which there is much diversity of opinion. This fact necessitates an attempt to formulate a theory of contraction which can embrace consistently the recorded morphologic data relative to striped muscle in the various phases of contraction and extension. It is recognized also that our theory concerning the intercalated discs of heart muscle has won only limited and generally qualified acceptance. Very recent revisions of text-books of general anatomy still describe heart muscle as composed of distinct cells, interpret the intercalated discs as cement lines, and ignore the histologic evidence that during contraction and extension some substance moves within the myofibril from mesophragma to telophragma, and in the reverse direction, respectively, as demonstrated by various staining methods. That the intercalated discs in cardiac muscle are not cement lines or cell boundaries is definitely proved by the fact that they do not extend completely through a fiber in the manner of a cell membrane, but are more or less deep peripheral structures. They do not extend centrally beyond the innermost myofibrils in cardiac muscle, and an occasional disc lies superjacent to a nucleus. The relative sparsity of typical intercalated discs in the heart of *Limulus* also contravenes any interpretation of adult cardiac muscle in terms of discrete cells.

Since our theory interprets intercalated discs of striped muscle as modified irreversible contraction bands, it is required that the precise morphologic features of contraction, which serve as the basis of the theory, be definitely established. It may suffice at this point to state that by 'contraction band' we mean the deeply staining area which appears on either side of the telophragma during muscle contraction (in contrast with a similar area on either side of the mesophragma when the muscle is in repose), as first clearly illustrated by Rollet (8) in his figure of the striped muscle of *Cassida equestris* (fig. 126, Jordan and Ferguson's Text-book of Histology). By 'contraction wave,' as seen, for example, in our specimen of the cat's esophagus, we understand a much wider irregular condensed area of adjacent groups of fibers; such 'waves' in any one limited area may in-

clude many 'contraction bands.' In Rollet's figure the 'wave' would include eight 'bands. An intercalated' disc is in its simplest condition (ontogenetically and phylogenetically) fundamentally identical, structurally and tinctorially, with such a contraction band. In mature heart muscle it has become secondarily modified through the influence of mechanical and probably chemical factors, and the increase of tissue fluid among the elements. Certain pathologic conditions (e.g., hypertrophy and atrophy) are characterized by specific varieties of discs.

DESCRIPTION

All of the fibers of the entire section, 15 mm. by 5 mm. in extent, have a fairly uniform structure. A deeply staining Q-disc alternates regularly with a lightly staining J-disc. These two discs are of approximately equal thickness. The Q-disc is bisected by a lightly staining H-disc. The resulting subdivisions of the Q-disc have in general approximately the same thickness as the dividing H-disc. However, there is some variation in the thickness of the H-disc in different fibers. The J-disc is bisected by a distinct membrane, the telophragma (fig. 1, *T*). A mesophragma (M-membrane) is only discernible in occasional small areas of several of the fibers. The Q-disc is colored dark purple; the J and H discs stain a light pink color; the telophragma takes a light purple stain. This description could apply equally well to a stretched fiber or to one at midphase of contraction. The available data are not sufficiently precise to warrant a final conclusion regarding the actual functional condition of the fiber, that is, whether contracting or passing into repose or stretched. All things considered, however, I incline to regard the muscle as fixed in a slightly stretched condition.

The illustration shows three intercalated discs—one isolated, two arranged in a pair. Very rarely a group of three or even four successive discs occurs. Nowhere in this section, barring groups of three or four discs, are the discs more numerous than indicated in this illustration; in general they are less closely spaced, the distribution varying in different portions of the section.

The discs consist of a row of bacillary elements in close lateral-juxtaposition, joined together through the middle by a telolophragma. These bacillary elements are portions of the included myofibrillae. The interbacillary tissue fluid takes a slightly

Fig. 1 Drawing of portion of longitudinal section of voluntary striped muscle fiber of human leg (formalin fixation, Mallory's phosphotungstic-acid-hematoxylin stain). *T*, telophragma (ground membrane, Z-line); *J*, clear (isotropic) disc; *Q*, dark-staining (anisotropic) disc; *H*, Hensen's disc; *I.D.*, intercalated disc. *T* stains lightly purple, *Q* and *I.D.* deeply purple, *J* and *H* light pink. The portion between the two nuclei was drawn by aid of a camera lucida; the portion below the lower nucleus, including the pair of intercalated discs, was added free hand from an adjacent fiber. Magnification 1500 diameters.

deeper stain than the substance of the H-discs or the uninvolved J-discs. Taken as a whole the discs resemble a double-comb structure, the 'bar' of the 'comb' being formed by the telophragma. The discs are more or less deeply placed peripheral structures. Careful focusing reveals the fact that they do not completely span the fiber. The isolated disc is typical; it is less dense laterally, showing thus the bisecting telophragma; a few discs are of equal and maximum density throughout.

Many of the discs are in the condition illustrated in the lower pair of discs; that is, drawn out for some distance on one side, this condition generally being confined to alternate sides of the discs in a pair. These discs are comparable to 'contraction bands' as defined above; as such they might be in late phases of contraction or early phases of extension, that is, in incomplete contraction or extension; or they might be contraction bands drawn out laterally by a stretching of the fibers. The stretching of the discs may have been the result of the relatively greater contraction on the part of adjacent portions of the unmodified fibers during fixation or normal function. If these 'discs' actually represented unmodified contraction bands, then we should expect to find them more abundantly and more extensively arranged in groups, the latter in lateral alignment in the form of contraction waves. In a contracting or relaxing muscle we should not expect to find the bands so generally isolated and so sharply localized. In view of these facts, it seems more likely that the discs have become modified through tension and that perhaps the entire fiber was in a stretched condition. Moreover, the fibers are frequently distorted, that is, sharply bent or drawn out in the vicinity of the discs, facts which indicate that the discs are levels of relative weakness.

In my earlier studies of the intercalated discs of cardiac muscle I have shown that the simplest type of disc, both ontogenetically and phylogenetically, is a band form bisected by the telophragma. Primary variations of this original type produce discs that are bounded on one side by the telophragma, and such as are bounded on both sides by telophragmata. Further secondary modifications resulting from the operation of mechanical

factors, including chiefly oblique tensions in consequence of the branched condition of the fibers of the heart musculature, lead to the various terraced forms. Hypertrophied muscle, characterized by longitudinally splitting myofibrillae, contains only serrated types. The discs of our specimen of leg muscle correspond, then, to the original band type with bisecting telophragma. In accordance with my hypothesis that intercalated discs are modified irreversible contraction bands, the discs bounded on only one side are interpreted as contraction bands only half of which became irreversible; those bounded on both sides by a telophragma as fused adjacent irreversible halves of successive contraction bands.

Viewing the fiber of the illustration as in midphase of contraction, we may more closely examine the isolated disc. The condition of this portion of the fiber may be indicated by the following formula: $T + J/2 + Q/2 + H + \text{Disc} (Q/2 + T + Q/2) + H + Q/2 + J/2 + T = 2 \text{ sarcomeres}$. In other words, the disc includes adjacent halves of successive Q-portions fused along the telophragma. The structure in question corresponds to a contraction band and to a simple type of intercalated disc. As such it consists of deeply staining, laterally aligned, portions of adjacent myofibrils bisected by a telophragma. Since these contraction bands are apparently irreversible as indicated by their localized distribution in the absence of contraction waves, and slightly modified as indicated by the cloudy appearance of the lateral 'stretched' portions, they would seem to be typical intercalated discs. As shown in a previous paper (6), the original discs (irreversible contraction bands) are modified in part also by the accumulation of tissue fluid in the intervals of the adjacent portion of the myofibrils as indicated by the considerable precipitation of silver nitrate in these locations. The occurrence of typical intercalated discs in voluntary striped muscle, as here described, fulfills the prediction made by deduction from the hypothesis constructed from the histologic data derived from the study of cardiac muscle, and to this extent it gives support to this hypothesis.

DISCUSSION

The above-outlined interpretation of the discs as modified irreversible contraction bands calls for a brief discussion of the histologic features of contraction. The histologic evidence shows that contraction and extension are accompanied by the passage of a deeply staining substance from the mesophragma to the telophragma and vice versa, respectively. This statement calls for evidence of the existence of a mesophragma in striped muscle. It must be admitted that such a membrane is under usual conditions not discernible with our present means of microscopic observation. In an earlier paper (4) on the leg muscle of the sea-spider, I showed that a mesophragma becomes discernible in the contracting portion of a fiber which has at the same time become stretched, because of the already fully contracted condition of one end of the fiber. Assuming that the mesophragma is too delicate to come within the limits of visibility under ordinary conditions, stretching of the fiber would result in lengthening the sarcomeres and in reducing their diameter, thus causing a relaxation of lateral tension and permitting the mesophragma to coarsen to a point where it comes within the limits of microscopic vision. This is in essence the idea of Heidenhain (3), who claims to be able to see a mesophragma even in human cardiac muscle. The behavior of the lightly stained portion of the Q-disc (i.e., the transient H-disc) under conditions of contraction and stretching points also to the presence of a dividing mesophragma in the Q-disc. The deeply staining portion of the Q-disc under these conditions divides exactly along a middle plane; such precise division of this disc could hardly occur so uniformly unless the disc were bisected by a true membrane. A small portion of at least one fiber of this section also shows a mesophragma with great clearness. This fiber is at midphase of contraction and the portion showing the mesophragma is apparently under some tension. That the mesophragma of a sarcomere, however, differs to some extent from the terminal telophragmata is indicated by the facts that festoons are not formed in the sarcolemma at its levels, the

myofibrils are not bent along its levels in distorted fibers, and the nuclear wall is not drawn out into ridges at its levels. This would seem to indicate that the mesophragma, in contrast to the telophragma, is not attached to sarcolemma, myofibrils and nuclear membrane. However, the essential difference between these two membranes may be simply one of relative strength or rigidity. The mesophragma may be sufficiently elastic to be able to yield without rupture to distorting forces, or it may be too delicate to withstand the strains that operate in fixed tissues to demonstrate the relationship between the telophragma and the myofibrils, sarcolemma and nuclear membrane.

It would seem, therefore, that any discussion of muscle contraction may confidently proceed upon the basis of the existence of two membranes, the terminal telophragmata of the sarcomere and the bisecting mesophragma. Since Merkel (7) first called attention to the 'reversion of the striae' during contraction, this phenomenon has been repeatedly described in many different specimens of striped muscle. It was especially well shown in the illustration of Rollet (8). Englemann (2) criticised this interpretation on the basis of his demonstration that, as revealed by the polariscope, the anisotropic substance of the Q-disc does not move during contraction, but remains permanently segregated more or less closely about the mesophragma. This conclusion is supported by the observations of Schaefer (9) on muscle treated with Rollet's gold chloride method.² Englemann (2) would seem to interpret the phenomenon of an apparent reversal of striae, as shown in stained sections, on the assumption that the absorption of the isotropic substance of the J-disc by the anisotropic substance of the Q-disc (upon which contraction is supposed to depend) causes a relative condensation of the sarcoplasm about the telophragma and thus produces a relatively deeper-staining area in this location. Schaefer (9), however, attributes the phenomenon to "the deeply moniliform

² It is now clear to me, however, that the sarcostyle of the wasp's wing muscle which Schaefer regards as in the contracted condition is one swollen and shortened by the action of the hypotonic formic-acid-water solution used in Rollet's technic.

shape of the fibrils" (p. 184), which he believes "tends to cause the constricted parts to appear dark." In other words, Schaefer interprets the apparent reversal of the striae during contraction as an optical illusion. He states further that "In alcohol preparations (both of the wing muscles and of the ordinary or leg muscle) in which the sarcous elements have been stained, there is no appearance of reversal of striation; the darkly colored sarcous element always occupies the central or bulged part of the sarcomere, and the unstained hyaline substance occupies the constricted parts of the sarcostyle" (p. 185). The latter statements seem to me in direct contradiction to the facts. In alcohol-fixed tissue stained with iron hematoxylin the striae do reverse during contraction, the stainable substance of the Q-disc moving towards the telophragma; and the deeply staining discs of contracted muscle, that is, the 'contraction bands,' are bisected by the telophragma, where the constriction, if any occurs, is uniformly located in contrast with the deeply staining band of extended muscle which is bisected by the mesophragma.

Furthermore, that the movement of material during contraction is not solely from the isotropic to the anisotropic substance, that is, from the telophragma to the mesophragma, but largely in the reverse direction is shown by the fact that the median portion of the Q-disc, the portion farthest removed from the isotropic substance, becomes pale first; this gradual paling or loss of tingibility passes distally in opposite directions from the mesophragma towards the telophragmata, and the myofibrils become swollen distally and assume a knobbed appearance as if material were actually accumulating terminally in the sarcomeres. These knobbed ends stain even more deeply than the general Q-substance of the relaxed fiber. This phenomenon was especially well shown in the sea-spider muscle and has been illustrated by various investigators on the histology of contracting muscle. If contraction only involved an absorption of isotropic by anisotropic substance, by reason of which the striae apparently reversed due to a change of relative density of the J and Q discs, then the terminal portion of the original Q-disc

should become pale first instead of the farthest removed portions (from the nearest point of alleged absorption) or the middle portion of the Q-disc.

To summarize, these are the fundamental histologic data upon which an adequate theory of muscle contraction must be based or with which it must harmonize, and which it must consistently embrace. The occurrence of a mesophragma and a telophragma as constituent elements of a sarcomere, the telophragma at least being intimately connected with the myofibrils, the sarcolemma, and, where a nucleus intervenes, with the nuclear membrane; changes, as evidenced by an alteration in staining capacity, within the myofibrils, these changes involving a movement of a deeply staining substance from the mesophragma to the telophragma, apparently without change in position of the anisotropic granules of the original Q-discs; these changes produce a 'contraction band,' that is, a deeply staining 'disc' bisected by a telophragma; such discs, failing to 'reverse' locally, correspond to the simplest type of intercalated discs of cardiac muscle, and may become further modified (physically, chemically and morphologically, involving the accumulation of tissue fluid) to form genuine intercalated discs; the appearance of contraction bands is coincident with a shortening and thickening of the myofibrils and of the muscle fibers and of the muscle as a whole; contraction and mechanical tension produce a comparable effect upon the Q-disc, a separation along the level of the mesophragma; when constrictions supervene in a contracted fiber they are at the levels of the contraction bands, that is, at the levels of the deeply staining portions, and are due at least in part to the fact that here the bisecting telophragma is attached to the sarcolemma, between two successive points of attachment to which the sarcolemma is festooned permitting thus a bulging of the intervening sarcous substance at this level.

The above-detailed facts are not in conflict with the newer theories of muscle contraction that attempt to explain contraction in terms of surface tension or electrical phenomena among the ultramicroscopic sarcous particles, in so far as these theories do not postulate an absorption of the isotropic sub-

stance of the J-disc by the anisotropic substance of the Q-disc or a passage of substance from the region of the telophragma towards the mesophragma. Both the change in structure and in staining reaction of the involved portions of the myofibrils demonstrate that the movement of material is largely in the reverse direction during contraction, from the mesophragma towards the telophragma. Such movement is coincident with contraction. How these two concurrent phenomena are fundamentally or causally related is a question whose answer lies outside the scope of the present investigation.

The chief aim of this investigation, finally, concerns a demonstration that genuine intercalated discs occur in voluntary striped muscle under certain conditions, and the further support of our hypothesis that the intercalated discs of cardiac muscle are in essence modified irreversible contraction bands. If the conclusion that these discs of human leg muscle are comparable with the intercalated discs of heart muscle is correct, as seems incontrovertible, then the latter discs can for an additional reason be no longer regarded as cell boundaries.

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Estudios sobre la estructura del músculo estriado.

V. Histología comparada de los músculos de las patas y alas de Mantis, con especial mención de los discos en N y los sarcosomas.

El disco en N es una estructura bien manifiesta en el músculo de las patas, pero no puede discernirse en el del ala. Está formado por elementos intrafibrilares y contribuye a la formación de la banda de contracción. Una banda de contracción comprende la substancia intensamente coloreable de las mitades opuestas de dos discos anisótrpos sucesivos, los dos discos en N intermedarios y el telofragma bisector. Los sarcosomas son cuerpos intrafibrilares (intersarcostílicos), de naturaleza lipoide en su mayor parte. Se agregan en ciertos estados de contracción formando grupos en forma de J y Q. Tanto los discos N como las bandas de contracción son esencialmente independientes de los sarcosomas. El telofragma actúa como una barrera efectiva que se opone al paso de los sarcosomas. Tal barrera no existe en la región ocupada comunmente por el mesofragma. En el músculo de las alas o de las patas de Mantis no existe mesofragma. El autor discute la relación de los sarcosomas con las mitocondrias. Las miofibrillas se continúan directamente con las fibrillas tendinosas.

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