Technical Papers

Macromolecular Arrangement within Muscle

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Electron microscopy has already given much information about the macromolecular components of striated muscle (*1, 4*). Thus it is shown that myofibrils of teased muscle are ribbons composed of parallel arrays of filaments associated with an amount of seemingly amorphous material that is greatest in the anisotropic regions.

For the present work, strips of psoas muscle separated from a rabbit at death were tied *in situ* to strips of wood at their resting length, then cut out and immediately fixed in formalin. Pieces of this fixed muscle were dehydrated by passage through alcohols, embedded in methacrylate, and thinly sectioned for electron microscopy by the procedures recently outlined by Neumann, Borysko and Swerdlow (*2*) and finally shadowed with gold-Manganin.

In favorable instances an astonishingly regular macromolecular arrangement can be seen in these sections. At a moderate magnification a section of a fiber cut nearly at right angles to the long axis will appear as in Fig. 1. The macromolecular filaments constituting the myofibrillar blocks are seen almost end-on as either dots or short rods. Their diameters correspond to those of the fila-

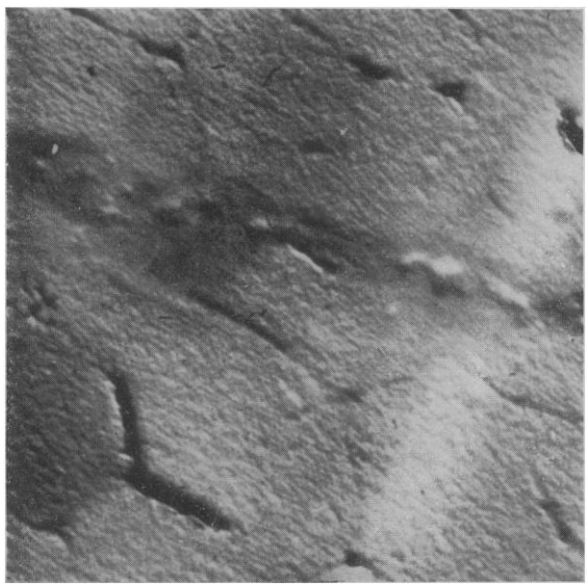

FIG. 1. A transverse section through a muscle fiber nearly normal to the fiber axis showing several myofibrils. Magnification, × 30,000.

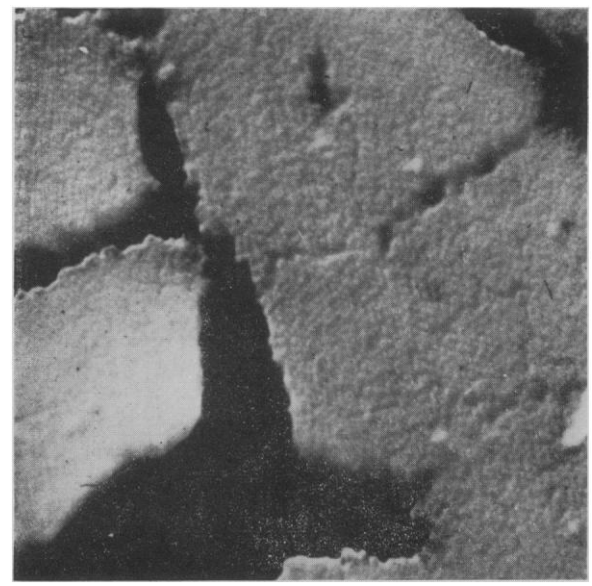

FIG. 2. A transverse section through muscle at a higher magnification. Magnification, × 50,000.

Though the study of such teased preparations has much to say about the macromolecular structure of muscle, it does not and cannot be expected to tell much about the relation between these thin strips or ribbons and the way they are built up in three dimensions in the intact myofibrils. This can be done only by the investigation of transverse and longitudinal sections. We are here reporting certain preliminary results of such an investigation.[2]

ments seen in electron micrographs of teased preparations. The order that is obvious in the arrangement of these macromolecules is clear at the higher magnification of Fig. 2. The section here is almost exactly normal to the fiber axis, the molecular net is approximately hexagonal, and therefore the filaments must be fairly close-packed in the fiber itself.

Longitudinal sections also have shown regularity in particle arrangement (Fig. 3). The macromolecular filaments, which run nearly vertical in this figure, have an obvious beaded structure and the beads of adjacent filaments are regularly aligned to give a net which might be rectangular if the section were cut exactly parallel to the fiber axis. The existence of such a net in longitudinal section, together with the hexagonal net seen in transverse

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[2] Electron micrographs recently published (*3*) of sectioned rat muscle have been interpreted to mean that the intact myofibril is a hollow cylinder or tube of which the teased-out ribbon is the shell or outer layer. As the accompanying photographs demonstrate, our results do not point to such a structure.

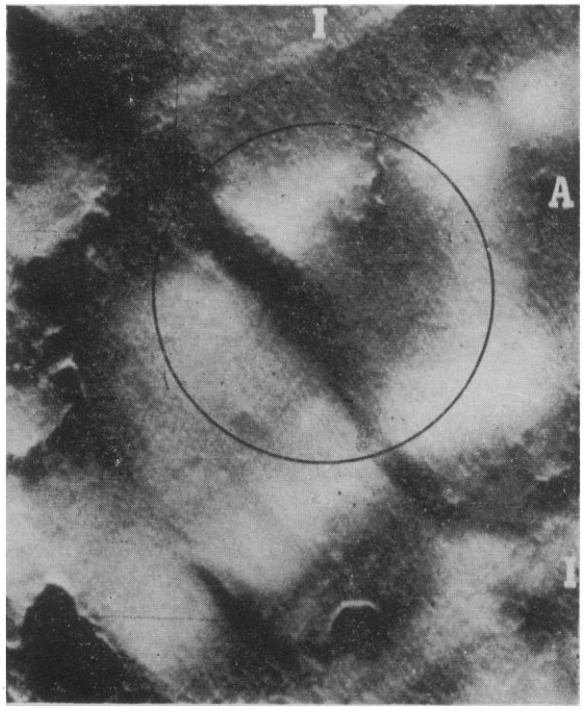

FIG. 3. A longitudinal section through part of a muscle fiber. Regions adjacent to I and A correspond to isotropic and anisotropic bands. Order in particle arrangement is best preserved in regions enclosed by the inked-in circle. Magnification, × 27,250.

section, demonstrates that there is three-dimensional, and by definition crystalline, order in the arrangement of the macromolecular components of the fibrils of this striated muscle. The regularity is most pronounced in the anisotropic bands.

An awareness of this high degree of order in its structure is obviously important for an understanding of muscle and of the way it functions. We are continuing this investigation to find out more about the nature and fine structure of the particles which display this order and to see how they are changed by muscular contraction.

References

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Synthesis of Greatly Enriched HD

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The maximum concentration of the hydrogen-deuterium molecule HD which can be obtained in the equilibrium mixture is approximately 50%. The equilibrium constant for the reaction $H_2 + D_2 = 2HD$ has been determined (*5*, *12*) and is near 4, which would be expected from random distribution of the three molecular species.

In making studies of the boron hydrides, I have found a method by which HD of 85% concentration can readily be prepared. The residual gases are H_2 and D_2.

The synthesis consists in direct complete deuteration of B_2H_6 by D_2, and slow reaction of the B_2D_6 with H_2O, producing HD. The reactions were followed and the gases analyzed by a mass spectrometer operated as has been described (*11*).

One other note of direct synthesis of HD was found. H. Beutler *et al.* (*2*) reacted LiH with D_2O, and used the ultraviolet absorption between 750 and 850 A to determine H_2, D_2, and HD. They report enrichment in HD above the equilibrium amount.

The first step in the B_2H_6 method is deuteration using D_2 gas obtained from the Isotopes Branch, U. S. Atomic Energy Commission. The deuteration proceeds slowly at room temperature, in a pyrex bulb, without catalyst. Equilibrium is attained in 1½ hr at 80° C, with only a small amount of thermal decomposition of the B_2H_6. Successive amounts of D_2 must be added, for the deuteration at each stage is only partial. This is due to the accumulation of H_2 removed from the B_2H_6 molecule in the process. After each stage in the deuteration, liquid N_2 was used to condense the B_2H_6-B_2D_6, the H_2-diluted D_2 was pumped away, and fresh pure D_2 was added. About seven stages, with large excess pressure of D_2 over B_2H_6 in each, produces B_2D_6 of purity greater than 95%, and further additions of pure D_2 give no further change in the mass spectrum of the B_2D_6.

The progress of deuteration was followed by the mass spectrometer. At complete deuteration, the ratio of mass peaks 32 to 31 is very close to 2.0. These peaks represent the ions $B^{11}B^{11}D_5$ and $B^{10}B^{11}D_5$. These values are to be expected from an abundance ratio of boron $B^{11}/B^{10} = 4.0$. This is in agreement with the value found at the National Bureau of Standards (*4*). The ratio of the parent ion, $B^{11}B^{11}D_6$ at mass 34 to the ion $B^{11}B^{11}D_5$ at mass 32 was found to be under 1%, even smaller than is the case with the parent ion for normal B_2H_6 and $B_2{}^{10}H_6$ (*3*, *11*).

The second step in producing highly enriched HD is to react the pure B_2D_6 slowly with low concentration of H_2O over H_2SO_4, at 25° C.

Into a 63-cc pyrex bulb, 5 ml of H_2SO_4, sp. gr. 1.752 (20° C) was placed. This is 82.8% H_2SO_4. It produces a water vapor pressure of about 0.03 mm at 25° C. The mass spectrometer showed no detectable SO_2 or SO_3 in the gas phase at this temperature. The acid was degassed by alternate freezing and thawing, and pure B_2D_6 con-