X-ray diffraction patterns from muscle

A preliminary study of the diffraction patterns from muscles of a marine invertebrate, using both low and wide-angle X-ray diffraction techniques, has been undertaken. The investigations were carried out on the retractor muscle of the sipunculid *Dendrostomum cymodoceae*[1] (a new species). The muscle appeared to contain only Type II fibrils[2] with an axial period of 420 A and a transverse period of 390 A. The wide angle pattern gives the usual α diagram and, also, a series of meridional reflections which are very well developed. The spacings of these reflections coincide with those of F-actin[3]. Crystalline material with imperfect orientation possibly related to the LOTMAR-PICKEN pattern[4,5,6,7] has been observed. These reflections disappeared after the muscle had been extracted with water. Similar behaviour of muscle specimens showing the LOTMAR-PICKEN pattern was found by previous investigators[6,7].

The diffraction apparatus and technique has been described in detail elsewhere[8]. The specimen to film distance was 22 cm for low-angle diffraction and 4 cm for the wide-angle camera which recorded spacings from 2.8 A to 30 A on a flat plate film. The X-ray specimen consisted of a single fibre as each of the two retractor muscles are of optimum thickness for X-ray diffraction using nickel filtered copper radiation.

The large axial and transverse periods were first investigated. The muscle was washed in distilled water for ½ hour and examined in the wet state. A fibre axis period of $d = 140$ A was obtained with an exposure of 2 hours. This reflection was quite sharp and free from the background scatter near the beam stop. This spacing agrees with the axial period $d = 420$ A given by HUXLEY[9] for living frog sartorius muscle assuming that the $d = 140$ A reflection is the 3rd order of the accepted axial period. The first order of this spacing would have been obscured by the rather intense background close to the beam stop due to continuous low angle scatter from the muscle. The first and second orders of the transverse period $d = 390$ A were resolved after an exposure time of three hours. The reflections in this case were comparatively diffuse diffraction maxima and the large period $d = 390$ A was the estimated average. This value agrees with the observation of HUXLEY[9] that living muscle consists of long molecules, arranged in an hexagonal array, 450 A apart.

Wide angle patterns taken in the wet state were somewhat unsatisfactory due to the large amount of water present. The muscle was pinned at normal length (the uncontracted state) and washed with distilled water for a few seconds only and then air dried which required about three days to complete. A typical pattern is shown in Fig. 1 from muscle which has been air dried for 2.5 hours. In addition to the muscle pattern, there are a number of partly orientated rings having a grainy texture in which reflections due to individual crystallites can be seen. All the reflections were to some extent arced on the meridian with the exception of a strong equatorial arc at $d = 4.86$ A. This crystalline pattern appeared only after the muscle was dried and the orientation continued to develop as the muscle dried. The orientation in the rings varied at different sites along the fibre although the same spacings were always observed. The spacings and estimated intensities of the reflections from this crystalline material are given in Table I.

TABLE I

SPACINGS OF REFLECTIONS WITH ESTIMATED INTENSITIES

Crystalline material Meridional Arc	*Muscle Meridional Arc*
5.97 vw	26.5 m
4.59 m	18.0 mw
4.26 mw	13.4 mw
3.68 s	10.8 s
3.15 m	9.05 s
3.07 m	7.75 mw
2.99 w	6.80 m
	5.76 vw
Equatorial Arc	5.10 vs
4.86 s	4.53 m
	4.29 mw
	3.89 w
	3.57 w

The spacings 5.97 A, 3.68 A, and 4.86 A also occur in the LOTMAR-PICKEN pattern. However, the first layer line reflection 4.86 A of the LOTMAR-PICKEN pattern was on the equator while the equatorial reflections occurred as meridional arcs. The other spacings given in Table I do not agree with the LOTMAR-PICKEN X-ray pattern.

The muscle which gave the pattern in Fig. 1 was washed in distilled water for four hours and air dried for four days. It then gave the diffraction pattern shown in Fig. 2. In addition to the usual α muscle pattern, it has a number of meridional reflections. The meridional spacings and their estimated intensities are given in Table I. These spacings, with the exception of the 5.10 A reflection of the α pattern, agree closely with those given by ASTBURY[3] for F actin. The only differences are that a very weak reflection at 5.97 A has been recorded, but none at 5.3 A and the actin reflections 3.1 A and 2.8 A described as very weak have not been detected in this muscle pattern. It may be

Spacings given in Angstrom units. Letters indicate intensities as follows: vs = very strong; s = strong; m = moderate; mw = moderately weak; w = weak; vw = very weak.

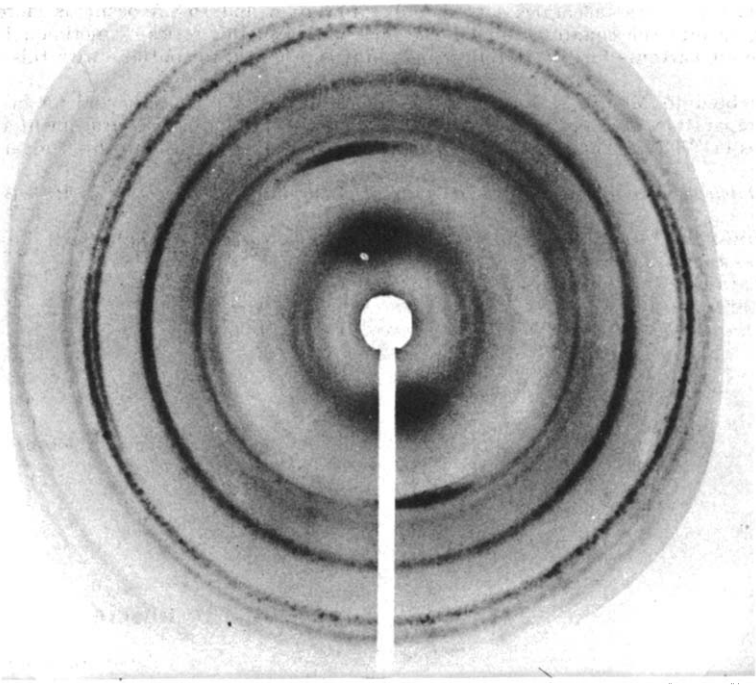

Fig. 1. A typical diffraction pattern from air dried muscle (× 1.7).

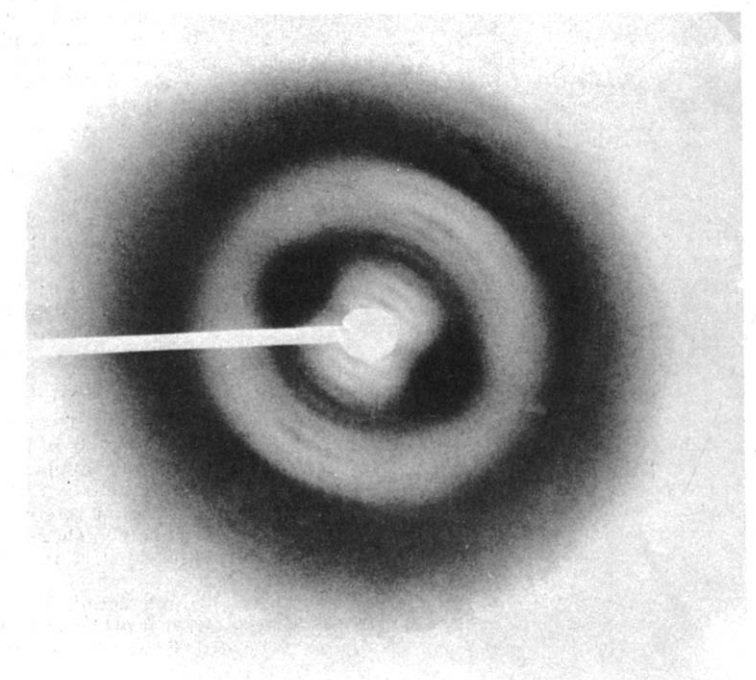

Fig. 2. Diffraction pattern after extraction with distilled water (× 1.7).

noted that the strong reflections corresponding to 9.05 Å and 10.8 Å occur as more extended arcs merging into the equatorial spots. The observation of this series of meridional reflections in such detail warrants further investigation and it is proposed to continue with this work.

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Heat stable protein from skeletal muscle

A substance having the general properties of a protein has been isolated from muscle by the following method:

Four-hundred grams of ground beef are stirred with 320 ml of 2 *M* $MgCl_2$ and 880 ml of water containing 12 ml of *N* HCl. The suspension, the pH of which should be 4.5, is heated to 90° and filtered hot. The filtrate is neutralized with NaOH and treated with 1 volume of methanol. The precipitate is sedimented and dissolved in 350 ml of 0.4 *M* $MgCl_2$ adjusted to pH 5.0. The solution is heated to 90°, the precipitate discarded. The supernatant is neutralized, the precipitate again discarded. The supernatant is dialyzed at 4° against 20 volumes of distilled water. The protein crystallizes in the form of thin needles or coarse sphenoids (Fig. 1). Crystallization is repeated by the same procedure. The yield is 0.4 g. The same method has yielded material of the same crystalline appearance when applied to human muscle.

The extraction of the protein was unsuccessful when NaCl or $BaCl_2$ was used instead of $MgCl_2$. Crystallization did not take place in the absence of Ca, Ba or Mg. $MnCl_2$ and $CoCl_2$ dissolved the protein but did not promote crystallization. Zn and heavy metals did not dissolve it.

Its solubility was approximately as follows: In the absence of salt, above pH 7 and below pH 4; in 0.02 *M* $MgCl_2$, only below pH 4; in 0.4 *M* $MgCl_2$, only above pH 5.

The crystalline preparations yielded a high ash (6.1 % when $BaCl_2$ was used for crystallization). This decreased to 0.25 % after dialysis against 0.01 *N* HCl. Nitrogen content was 14.0 %, carbon 42.6 %; phosphorus and sugar were not detected.

The viscosity of solutions in 0.4 *M* $MgCl_2$, pH 7.0, was measured at 30° in Ostwald type pipettes and yielded an intrinsic viscosity $[\eta] = 0.38$. Hence[1], Simha's factor $\nu = [\eta]\ 100/\bar{v} = 51$ (assuming 0.74 for the partial specific volume $\bar{v}$); hence[2], an axial ratio of 24:1.

The protein was homogeneous in the ultracentrifuge in 0.4 *M* $MgCl_2$, pH 7.0, and yielded $s = 2.7 \cdot 10^{-13}$ at infinite dilution. From this and the axial ratio, with the help of the simplified form of Perrin's equation[3], the following values were obtained: radius of an ellipsoid of revolution, $b = 1.03$ mμ; semi long axis, $a = 25$ mμ; molecular weight = 91,000. In 0.01 *N* HCl, pH 2.5, ultracentrifugation revealed two components, whose sedimentation rates at zero concentration appeared to be of the order of magnitude of 2 and $3 \cdot 10^{-13}$ very approximately. The curve relating s to concentration was, however, so convex toward the origin that no satisfactory extrapolation could be made.

The substance shares with the other muscle proteins, myosin[4], actin[4], and the peptomyosins[*,5,6], the property of being insoluble in high salt concentration at pH below 4. It resembles peptomyosin B[6] in molecular shape, being slightly less elongated. However, since the sedimen-

[*] Peptomyosin A also is insoluble below pH 4 in *M* NaCl. This fact was not stated in the original description.