10,000×. The DNP solution contained fibrous elements, preferring to use a term of DNP fibres[5,6].

In order to prepare hypoxic DNP solution, about 3 ml of the DNP solution in a Pyrex glass tube (17 cm³) were kept at 0 °C, and bubbled with nitrogen gas (99.95%) through a fine needle at a flow rate of 100 ml per min for 15 min. The hypoxic DNP solution thus prepared revealed essentially the same configuration of the DNP fibres as that of the DNP fibres observed in the aerobic condition (Figures 1 and 2). The DNP fibres, about 2,100 Å in diameter, were formed by a coiling of subfibres with about 850 Å diameter (Figures 1 and 2, arrows), as previously reported by NAKANISHI et al.[6].

The DNP solution under aerobic condition was irradiated with 10 krad and 1 Mrad at dose rates of 800 rad and 15 krad per min, respectively. Remarkable alterations of the DNP fibres in both the irradiated samples were a reduction of their diameter, about 800 Å in diameter (Figures 3 and 4). In some places, 2 thin fibres were closely contacted, and run parallel, representing a tape-like wide structure (Figure 4, thick arrows). The evidence suggests that the DNP fibres are extended by irradiation under aerobic condition, resulting in the formation of the thin fibres. Furthermore, it was observed that the aggregates and ring-shaped loops were constructed by the thin fibres, the former being indicated by black arrows and the latter by white arrows in Figures 3 and 4, respectively.

On the other hand, when the hypoxic DNP solution was irradiated at a dose rate of 15 krad per min giving a high dose of 1 Mrad, no apparent change of the DNP fibres was demonstrated in their structure, except that the surface of the DNP fibres exhibited rough appearance (Figure 5) and that the ring-shaped loops were found to be formed (Figure 6, arrows). The image seems to be more similar to that of the DNP fibres observed in the non-irradiated aerobic and hypoxic DNP solutions than to that of the DNP fibres irradiated with 10 krad and 1 Mrad under aerobic condition.

Although a final conclusion must be left to future studies, it seems probable that the different appearances of the DNP fibres are affected by the presence or absence of oxygen during irradiation. Further detailed and quantitative studies on the fine structure of the DNP fibres under various conditions are now in progress[7].

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Organization of Myosin Molecules in the Muscle Thick Filament

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Summary. Tryptic treatment of muscle thick filaments reveals the underlying backbone of aggregated L-meromyosin as a coil of 3 secondary filaments (helical repeat ~130 nm) each in turn a coil of 3 finer ones.

The cyclical interaction of the myosin heads of thick filaments with the g-actins of the thin filaments is at the basis of muscular contraction[1]. This interaction appears to involve subtle steric orientations of the heads and as such, the precise organization of myosin in the thick filaments is an important aspect of the contractile mechanism[1,2].

The reflections from low angle X-ray diffraction of pre-rigor vertebrate muscle are interpreted as showing that the thick filaments have their myosin-head projections arranged at successive levels of about 14.5 nm along the filament backbone[3]. The heads are in a helical array described severally as two-stranded 6/1 of pitch 86.4 nm[3]; four-stranded 6/1 of pitch 86.4 nm[4]; three-stranded 9/1 of pitch 129.6 nm[5]. The values 6 or 9 refer to the number of projections in each helical turn of each strand.

Further advance in resolving the molecular organization is likely to come from electron-microscopic identification of key details not shown by X-rays. We report pertinent new structural features revealed by controlled tryptic digestion of thick filaments to remove the H-meromyosin moiety, and to expose the underlying aggregated backbone of L-meromyosin.

Hen pectoral muscle was used to prepare natural thick filaments[6] and the myosin for forming synthetic filaments[7]. Digestion was carried out in two ways. Trypsin, 10 mg/ml in 0.1 *M tris*-HCl, pH 7.6[8] was added in equal volume to dilute suspensions of the filaments. After digestion, droplets of the suspensions were applied to carbon-coated grids. Alternatively the trypsin (5 mg/ml) in the same buffer was added as droplets to grids on which the filaments had already been lodged. In both cases digestion at 20 °C was continued for 40 min. Head removal was incomplete at lower trypsin concentrations or shorter digestion times. Undigested and digested filaments on the grids were then fixed for 5 min in 1% glutaraldehyde, 2 m*M* imidazole-HCl, pH 7.0. Negative staining was carried out with 1% uranyl acetate[9].

Although both methods revealed the same structures, the Figure illustrates those obtained by digestion in the suspensions. The untreated natural (a) and synthetic (b) filaments, as expected, were covered in projections except for relatively smooth central regions. The roughly oblong

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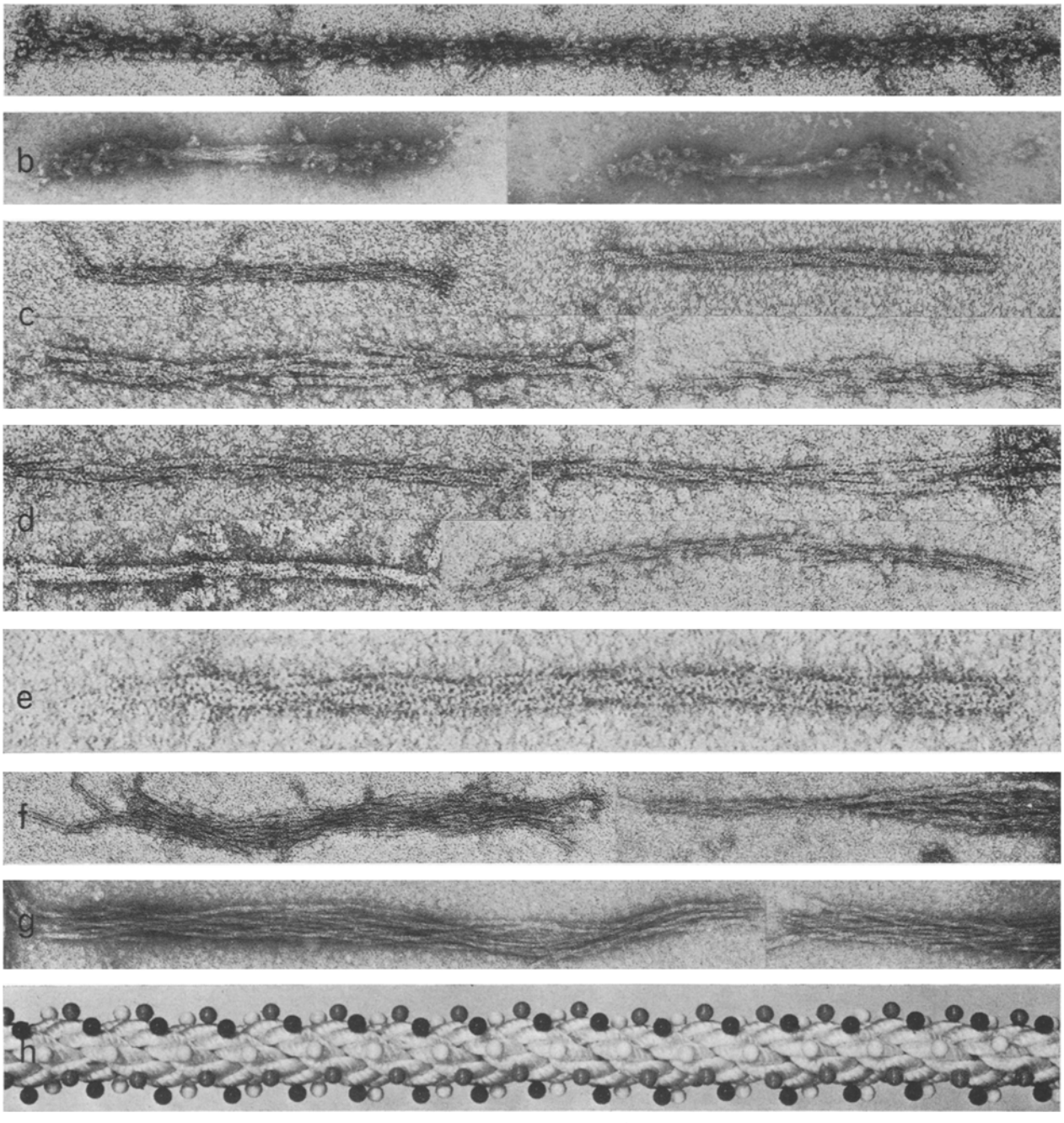

a) Natural thick filaments studded with heads and showing signs of a bare central zone. × 100,000.

b) Synthetic thick filaments. Although of less than average length they are included for their clearly defined bare zones and regions of projections. In some cases the projections are cleaved, representing either the two parts of the myosin head, or head pairing. × 100,000.

c, d) The coiled backbone of fragments of natural (c) and synthetic (d) thick filaments revealed by tryptic digestion. × 200,000. The helical repeat of each strand is ~130 nm, the helix angle ~80°.

e) One of the natural backbone filaments shown in (c) further enlarged. Very clear coiling is seen by viewing it along its length at eye level. Some coiling in the secondary strands is apparent. The superimposition of the top and bottom surface patterns gives the filament a plaited appearance. × 370,000.

f, g) Portions of intertwined bundles of ~9 primary filaments produced by tryptic digestion of natural (f) and synthetic (g) filaments. These are presumed to be loosened examples of the coiled structures seen in c and d. The pieces on the right are tasselated ends of the filaments and show some fine coiling. × 100,000.

h) Proposed model of the studded regions of natural and synthetic thick filaments. The beads are the probable points of emergence of the H-meromyosin end of each molecule which are all equivalent in the structure. The beads are also a reflection of head array and take up the precise helical, three-stranded 9/1 arrangement which is one of the limited possibilities demanded by X-ray diffraction of striated muscle. In the model the helix angle is less than in the filaments. They would have been the same if cord representing the secondary strands had had the correct helix angle. Superimposing the mirror image of the model on itself would give the same sort of plaited appearance seen in (c).

heads of the natural filament were rather more distinct than has previously been described[6], but there was insufficient order to determine molecular organization. In contrast, tryptic treatment removed the heads to reveal considerable underlying detail. The most common components were the coiled structures (diameter ~ 13 nm) seen in the micrographs of treated natural and synthetic filaments (c–e). Top and bottom surface patterns are superimposed in the negatively-stained filaments. However their general appearance and seeming three-stranded nature is strong evidence for their consisting of 3 fibres (diameter 6–7 nm) wound with what appears as a right-handed screw, helix angle ~80°, helical repeat ~130 nm.

Another structural species observed in both natural (f) and synthetic (g) filament preparations were loose bundles of 8–10 fine strands held together by a high degree of intertwining. We consider these to be loosened forms of the tight coils seen in c–e. The diameter of these fine strands is 2–3 nm.

Although a left-handed coiling is evident amongst them neither this nor the helical repeat was able to be determined with certainty. The coiled structures are not artefacts formed from the digested material since the same forms are produced by digestion of thick filaments already attached to the grids.

The present information, by giving insight into the molecular organization of the backbone, restricts further, possibilities for head arrangements. It seems most probable the backbone is a triple coil, with a helical repeat of 130 nm. This strongly favours SQUIRE's[5] three-stranded 9/1 model over the 2- and 4-stranded arrangements. Further weight is given to this design, since seemingly 9 of the finer filaments make up the structure, presumably combined in 3 sets of 3 coils.

The information on coiling and filament numbers can be used in model building. Considering only the studded regions of the filament, the model shown in Figure h) brings together the present information and the X-ray data. In the model, the rope-like backbone is a right-handed tertiary coil of three secondary ones. Each of these in turn is a left-handed triple coil of 3 primary strands. The beads on the model surface are included to highlight the ordered relationship between strands. They are the likely points of emergence of H-meromyosin tangentially to the secondary filaments, and, depending on the coiling in the fine tertiary strands, closely in line with the model backbone. Alternative coilings are possible. For instance, if the secondary and tertiary strands in the model are wound in the same sense, the H-meromyosins would emerge approximately at right angles to the filament axis.

Without resorting to rigid proof, it is evident from the model that a strict relationship exists between the helical repeat (~130 nm) in the tertiary coil and the repeat in the triple coil of the secondary. In this respect, on winding together the 3 secondary filaments in the model, the 9/1 arrangement is achieved at the point at which resistance to further winding is first felt. This is because, with additional winding in the tertiary coil, the secondary coils are being forced to unwind.

On the basis of the model, the primary strands in the thick filaments are presumed to be formed from myosin molecules overlapping in the L-meromyosin region. Their rather regular width also favours coiling as this would hinder further side by side aggregation expected with strictly parallel alignment[10,11]. Assuming the various filaments to be circular in cross section, the 13 nm diameter of the backbone would reduce, by calculation to a diameter of the myosin tail of ~1.3 nm, rather less than the 2 nm that has been reported[12].

The proposed coiled structure gives equivalence to all the myosin molecules in the studded regions, without the need to propose a central supporting core of another protein[5]. The fact that synthetic filaments from the purified myosin form the same basic structures as the natural filaments, supports the non-existence of a core substance. A comprehensive report on the coiled nature of the thick filaments will appear later.

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Gene Amplification in Oocytes of the Rove Beetle *Creophilus maxillosus* (Staphylinidae, Coleoptera-Polyphaga)[1]

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Summary. The extra-DNA body was found, for the first time, in oocytes of an insect with telotrophic ovary. Dispersion of this body is accompanied by an enormous increase of nuclear volume and production of multiple nucleoli. It is suggested that the extra-DNA contains a huge mass of nucleolus organizers.

It is known from experimental and descriptive studies that, at a given degree of ploidy, there is a close relationship between the size of an interphase nucleus on the one hand, and decondensation of chromatin and intensity of RNA-synthesis on the other[2,3]. This relationship seems to occur also in the prophase nuclei of growing oocytes. Thus, in insects with polytrophic or telotrophic ovaries, i.e. in the case when the growing ooctye is supplied with RNA produced by nurse cells, the oocyte chromosomes form more or less compact karyosphere, the nucleus is most often relatively small and RNA-synthesis in it is decreased in the same measure as its chromosomes are condensed[4–13].

The behaviour of oocyte nuclei deviating from the rule of restricted RNA-synthesis in the polytrophic ovary is conditioned by the presence of a considerable quantity of extrachromosomal DNA[6,14]. In such cases, inspite of the fact that the oocyte chromosomes form a karyosphere, the volume of the nucleus increases considerably and the nucleus is very active in the process of RNA-synthesis.