lecithin and water is 13.9 according to Katz and Diamond[18]. At local anaesthetic levels, the benzyl alcohol–dimyristoyl lecithin mol ratio is 0.04–0.11. As may be seen from Fig. 1*a* there is no decrease in the electric quadrupole splitting from the pure lipid value of 26.2±0.5 kHz in this range of benzyl alcohol concentration, a result consistent with spin-label studies[9], although on a different timescale and without the possible complications of a perturbing probe effect.

At higher benzyl alcohol–dimyristoyl lecithin mol ratios there is a decrease in quadrupole splitting (or order parameter) with increasing concentration of benzyl alcohol, which agrees with spin-label studies[9] (Fig. 1). From these results it is relatively straightforward to determine quantitatively the projections of each C–C segment onto the bilayer normal[10,13,17], and so determine the effective length of the hydrocarbon chain, or the total membrane thickness[10,13,17,19]. Calculated chain lengths are rather insensitive to chain tilt. For example, for pure dimyristoylphosphatidylcholine at 60 °C, the distance from C-2 to C-14 is 10.2(6) Å assuming no chain tilt, or 10.5(2) Å assuming a gaussian distribution of chain orientations with a probable tilt angle of 25° (refs 10, 17). Similar average chain lengths are obtained using flat or lorentzian distribution functions (E.O. and R. Jacobs, unpublished). Consequently, we obtain from the NMR data a model-insensitive value for the average chain length, or membrane thickness. These lengths are in excellent agreement with those determined using high-resolution neutron diffraction (D. Worcester, M. Meadows, D. Rice and E.O., unpublished). For example, 23 °C lecithin–cholesterol data give a total hydrocarbon region thickness of about 30–31 Å, and neutron diffraction measurements[10] indicate a thickness of about 33 Å (Table III of ref. 10). From the data in Fig. 1*b* and *c* we can calculate the thickness of the dimyristoylphosphatidylcholine membrane at 38° C and obtain values of about 2.5(0) nm for the hydrocarbon region thickness in the absence of benzyl alcohol and about 2.3(6) nm in the presence of benzyl alcohol. The fluidisation of the bilayer caused by benzyl alcohol[8,9] decreases the membrane thickness. Cholesterol (30 mol%) causes an increase in hydrocarbon chain order, corresponding to about a 0.46-nm increase in membrane thickness at 23 °C (ref. 10).

Previous workers[5,6] have found, using black lipid membrane capacitance and conductance measurements, a 1.2-nm increase in membrane thickness in the presence of benzyl alcohol, and a decrease in hydrocarbon region thickness in the presence of cholesterol. Although the fatty acid composition of the lecithin used in the black lipid membrane studies differed slightly from ours, neutron beam and X-ray diffraction measurements on the egg-lecithin–cholesterol system indicate no significant decrease in membrane thickness on addition of cholesterol[21–23]. Similarly, other ^{2}H NMR and electron spin resonance spin-label studies of egg lecithin and egg-lecithin–cholesterol systems indicate an ordering of the hydrocarbon chains by cholesterol[15,24,25], which must correspond to an increase in projected chain length. In the absence of hydrocarbon solvents, such as tetradecane, an increase in projected chain length must correspond to an increase in membrane thickness, as neutron and X-ray data rule out ≥0.2 nm-chain interdigitation.

We therefore suggest that the previous black lipid film results[5,6] may be attributable to the inclusion of variable concentrations of solvent tetradecane in the lipid membrane, the exact amount being a function of the amount of cholesterol or benzyl alcohol present.

Deuterium nuclear magnetic resonance results suggest that the direct effect of benzyl alcohol on membrane bilayer thickness is negligible at the anaesthetic levels commonly used in clinical studies. At much higher levels (corresponding to an ~30–80-fold overload) there are small (≈0.1 nm) decreases in membrane thickness which are consistent with recent X-ray and neutron-beam studies of the effects of gaseous general anaesthetics on lipid bilayer thickness[26]. Taken together, our results cast considerable doubt on the importance of membrane thickness changes as a factor in the mechanism of action of many anaesthetics.

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X-ray diffraction patterns from molecular arrangements with 38-nm periodicities around muscle thin filaments

A SERIES of meridional and near-meridional reflections have been observed in X-ray diffraction patterns (ref. 1 and Fig. 1*a*) from the crab *Plagusia* leg muscle in the living, resting state, and have been attributed to troponin molecules which lie in pairs on thin filaments at 38-nm intervals. In the pattern (ref. 1 and Fig. 1*b*) obtained from the same muscle in rigor, the corresponding series of reflections was observed to have stronger intensities and higher order reflections. The reflections were interpreted as arising from both troponin and the cross-bridges. Electron micrograph[2] showed that cross-bridges occurred in symmetrical pairs around the thin filaments with a repeating distance of 38 nm in which one or a few closely spaced pairs may be included. Recently, reflections indexed as orders of 76 nm in X-ray diffraction patterns from insect flight muscle in rigor[3] and those from scallop striated muscle in rigor[4] have also been interpreted in terms of the cross-bridges attached in pairs to thin filaments with a repeating distance of 38 nm. We report here a method that enables us to analyse diffractions generated by molecular arrangements of this type. These arrangements are described by a set of identical helices, and the systematic modulation of intensities, indexed as orders of 76 nm, is interpreted in terms of displacement between these helices.

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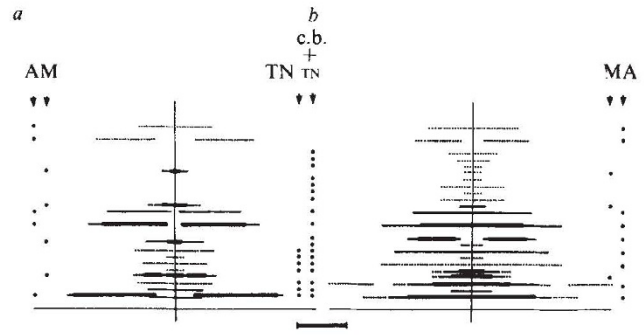

Fig. 1 Schematic representation of X-ray diffraction patterns[1] from crab *Plagusia* leg muscle in the living resting muscle (*a*), and in rigor (*b*). In addition to actin layer lines (marked by A) and meridional reflections (marked by M) attributed to myosin projections on the surface of the thick filaments, there is a series of meridional and near-meridional reflections (including intensities observed at $<0.07\ nm^{-1}$ on some of the actin layer lines) which were indexed as orders of 2×38 nm. In *a*, the reflections (marked by TN) were seen up to the 9th order, whereas in *b* those (marked by c.b.+TN) were observed up to the 24th. Thick solid lines, solid lines and dotted lines represent intensities observed even on the third film, up to the second one and on only the first film of each set, respectively. Bar, $0.1\ nm^{-1}$.

The thin filaments consist of actin, tropomyosin and troponin in a molar ratio of 7:1:1. The actin helix has a symmetry of 28/13 (28 molecules are included in 13 turns of the short-pitch helix) in the crab leg muscle in rigor. If we assume that these protein molecules interact with each other at specific sites, then the arrangement of troponin can be described by two identical 2/1 helices as shown in Fig. 2*a*. Axial displacement (Δz) and azimuthal rotation ($\Delta\phi$) between two molecules in pairs are specified by k multiple of those values between adjacent two-actin subunits along the 28/13 actin helix, where k is an odd integer. Ohtsuki[5,6] proposed a plausible model with $k=1$, which remains to be proved.

Uniformly spaced layer lines, indexed as orders of 76 nm, are predicted from the single 2/1 helix as shown in Fig. 3*a*. Every even (odd) order Bessel function contributes to every even (odd) order layer line. The addition of another 2/1 helix results in a systematic intensity modulation which depends on k. As there is no trace of lattice sampling on the layer lines in the actual resting and rigor patterns from the crab leg muscle, we propose that the cylindrically averaged intensity[7] on each layer line can be written as

$$\langle F(R)F^*(R)\rangle_l = \sum_n J_n^2\ (2\pi Rr)|a_l^n|^2 \qquad (1a)$$

$$a_l^n = \sum_j \exp i\{-n\phi_j + 2\pi l z_j/c\} \qquad (1b)$$

where J_n is the nth order Bessel function, a_l^n the modulation term, and r, ϕ_j and z_j radial, azimuthal and axial coordinates of a molecule on the jth helix. In equation (1*a*), the effect of shape and size of a subunit on the intensity is not included. The summation in equation (1*a*) is taken over n, satisfying the selection rule[8] of $l=n+2m$, where m is an integer. As $\Delta\phi=(1-1/14)\pi k$ and $\Delta z=c/28\times k$, then $|a_l^n|^2=4\cos^2\{\pi k/28\times(l+n)\}$ for $l=$ even and $4\sin^2\{\pi k/28\times(l+n)\}$ for $l=$ odd. If $k=1$ is assumed, even order Bessel terms would be dominant around the equator and $l=28$, and odd orders would be dominant around $l=13$ and 15 as shown in Fig. 3*c*, because modulation terms for meridional J_0 reflections (near-meridional J_1+J_{-1} reflections), for example, gradually vary with even (odd) values of l. If $k=3$ is assumed, modulation terms vary three times faster with l (Fig. 3*d*).

In the actual resting pattern from the crab leg muscle (ref. 1 and Fig. 1*a*), (1) reflections are observed at every order from 2nd to 9th, except at 5th, (2) even order reflections have intensity maxima on the meridian whereas odd orders have not. These features are well explained only when $k=1$. The presence of a meridional reflection at $l=4$ and near-meridional one at $l=9$ are hardly explained by $k=3$. $k=5$ and 7 are also excluded. The near meridional reflection at $l=5$ may be buried in the strong reflection at 14.4 nm (assigned to thick filament). In the muscle in the resting state, the actin helix symmetry is slightly different from 28/13, and accordingly, the troponin

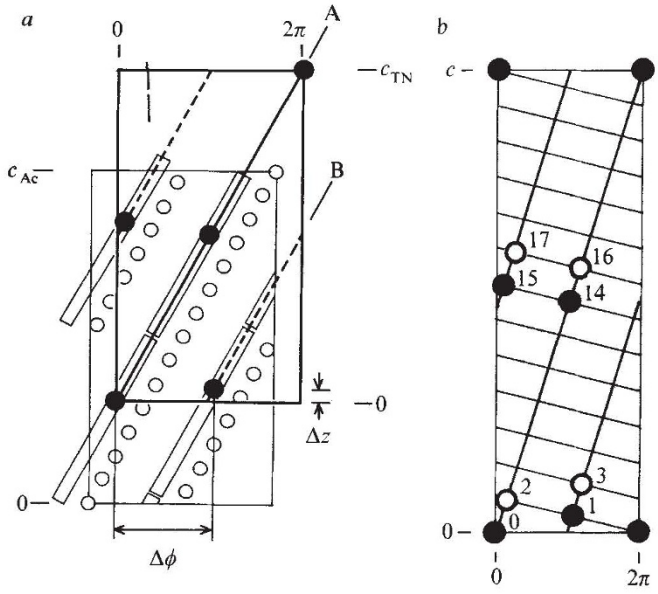

Fig. 2 *a*, The radial projection of the thin filament in Ohtsuki's model. A troponin molecule (closed circle) is attached to a specific site on a tropomyosin molecule (rectangular) which interacts specifically with seven actin subunits (open circles) arranged in a helix with the symmetry of 28/13 and the repeating distance of c_{Ac}. The arrangement of troponin molecules consists of two identical 2/1 helices, A and B, with the repeating distance of $c_{TN}=c_{Ac}$. Axial displacement (Δz) and azimuthal rotation ($\Delta\phi$) between two helices are specified by k multiples of those values between adjacent two actin subunits along the 28/13 actin helix, where k is an odd integer. Ohtsuki's model assumed $k=1$. In the crab leg muscle[1], the symmetry of the actin helix is 28/13 in rigor, but in the living resting state it is between 26/12 (= 13/6) and 28/13. This means that the troponin helix differs slightly from 2/1 in the resting muscle. The solid frames represent one repeating unit of the structure. The shape of each molecule and the precise relationship between them are not shown. *b*, The radial projection of two possible models for the cross-bridge arrangement around the thin filament in crag leg muscle in rigor. When a pair of cross-bridges ($L=1$) occurs symmetrically around the thin filament at intervals of 38 nm, four lattice points (closed circles) out of 28, labelled from 0 to 27 along the genetic helix, are occupied by cross-bridges. This arrangement is the same as the troponin arrangement shown in a ($k=1$). When two pairs ($L=2$) of cross-bridges occur at positions adjacent to each other, four more sites (open circles) are occupied. In the latter case, the arrangement can be described by four identical 2/1 helices; each helix includes molecules labelled 0 and 14, 2 and 16, and so on.

helix is deformed slightly from 2/1. This difference is too small to affect the diffraction patterns[2]. We conclude that the troponin arrangement in Ohtsuki's model is valid for the thin filaments of the crab leg muscle, illustrating an application of this method in which intensity modulation is interpreted in terms of helical arrangement of molecules about the thin filament axis.

If we assume that the head portions of myosin molecules are specifically attached to actin subunits, the surface lattice of cross-bridges around thin filaments in rigor (Fig. 2*b*) is the same as that of troponin molecules with $k=1$, namely, cross-bridges occur in pairs at intervals of 38 nm. When another pair comes to the next position and both pairs are included at every 38-nm interval (Fig. 2*b*), the surface lattice can be described with four

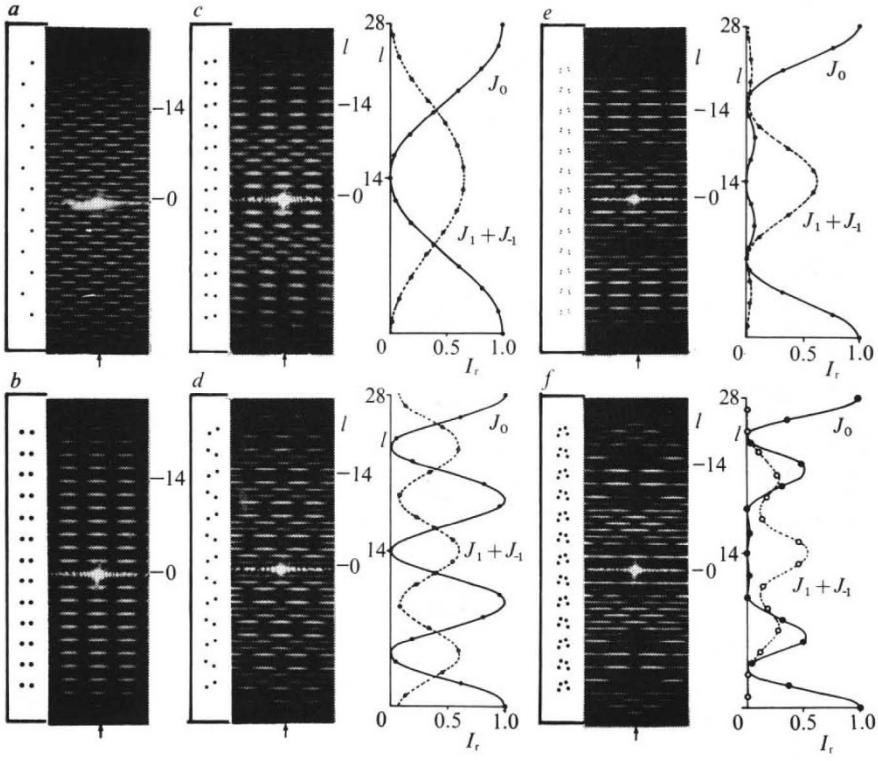

Fig. 3 Models of molecular arrangement around the thin filament and intensity modulations of diffraction patterns predicted from them. Models are *a*, single 2/1 helix; *b*, a pair of subunits in every 38 nm. The arrangement can be described with two identical helices with $\Delta z = 0$ and $\Delta\phi = 0$. *c*, The same as in Fig. 2*a*, *b* (closed circles only). The arrangement can be described with two identical 2/1 helices with $k = 1$. *d*, Two identical 2/1 helices with $k = 3$. *e*, The same as in Fig. 2*b* (closed circles plus open circles). *f*, Offer and Elliott model[10] for the arrangement of cross-bridges in insect flight muscle in rigor. In every 38 nm two pairs are attached to positions next but one to each other. In each set, the left is a model. Closed circles represent troponin and cross-bridges. The right of *a* and *b*, and the middle of *c*–*f* show optical diffraction patterns obtained from these models. To make higher order layer lines visible, the size of the circles in every model is chosen as that in *e*. The meridian is indicated by an arrow in each pattern. On the right in *c*–*f*, relative intensity maxima (I_r) of J_0 and J_1+J_{-1} contributions are plotted against *l*. These are calculated by equation (1), and the contribution of the form factor is neglected. In reality, unity in intensity in *e* and *f* is stronger by a factor of 4 than that in *c* and *d*. The necessary exposure time for the optical diffraction pattern in *a* is then 4 times as long as those in *b*, *c* and *d*, and 16 times as long as those in *e* and *f*. Note that if two molecules in pairs are axially aligned as in *b*, no odd order reflections can be observed. All the models are based on the actin helix of 28/13. Although the actin helix is slightly different in the crab leg muscle in the resting state, nevertheless, the difference is so small that the diffraction patterns are not affected[2].

identical 2/1 helices. When the number of pairs is increased to L, modulation terms, for example, for meridional J_0 reflections calculated by equation (1) are given as

$$|a_l^{n=0}|^2 = \sin^2(2L\pi l/28)/\sin^2(\pi l/28) \qquad (2)$$

which is an example of Laue function (Fig. 3*e*). Note that the more cross-bridges there are, the smaller the number of reflections on which intensities become concentrated. In the actual pattern from the crab leg muscle in rigor (ref. 1 and Fig. 1*b*), (1) meridional and near-meridional reflections were observed at orders from 2nd to 24th, of 76 nm; (2) meridional reflections at $l = 12$, 14 and 16 are absent, apparently due to a selection rule (if the form factor is diminished there, we cannot explain the fact that near-meridional components at $l = 13$ and 15 are observed in the actual pattern); (3) even order reflections have intensity maxima on the meridian, whereas odd order reflections have not. These features can be explained both by the model with $L = 1$ and by that with $L = 2$. In the diffraction pattern predicted from the $L = 2$ model, a few reflections (especially at $l = 2$) would be almost four times stronger and the rest would be weaker than in the $L = 1$ model. Nevertheless, we cannot decide between the two without measuring intensity distribution. Models with $L \geqq 3$ predict extinction of a meridional reflection at $l = 4$ in disagreement with the actual pattern. Any other model can be excluded. As we have pointed out[1], troponin molecules also contribute to these reflections. However, the result is not altered by the troponin contribution in this qualitative analysis of intensity modulation, as troponin does not make an important contribution to these intensities; the ratio of intensity of reflection in resting pattern to the corresponding one in the rigor pattern is smaller than 1/4 and higher order ($l > 9$) reflections are not observed in resting patterns.

To decide between the two possible models, and to determine the spatial relationship between troponin and cross-bridges on the same thin filament and also the size and shape of each molecule, we must make the analysis quantitative by obtaining a cylindrically symmetrical Patterson function[9].

Offer and Elliott[10] have proposed a model for the cross-bridge arrangement of insect flight muscle in rigor. In this model, two pairs of cross-bridges are included every 38 nm, but the second pair is positioned next but one to the first pair (Fig. 3*f*). The intensities predicted by the model, again based on intensity modulation calculated by equation (1), seem to be consistent with the recorded X-ray diffraction pattern[11]. In this muscle, the thin filaments are themselves arranged in a helical manner about the thick filament[12,13]. The absence of meridional reflections at $l = 2$ and 4 may be explained by the large helix. Lack of reflection at $l = 3$ might support the model, although other possibilities cannot be excluded. The disappearance of several near-meridional components of the diffraction pattern from the muscle after immersion in S-1 solution[3] could well be explained by the occurrence of more cross-bridges on the surface lattice with a symmetry of 28/13.

Vibert *et al.*[4] have recorded a series of reflections from scallop striated muscle in rigor which are thought to be similar to those from crab leg muscle in rigor. They observed an increase in intensities of near-meridional components at 38 nm and at 19 nm, after removal of the regulatory light chains. We might explain these observations by a rearrangement of the cross-bridges: from the arrangement with $L = 1$, for example, to that with $L = 2$. If this hypothesis is true, the intensities of some reflections would be decreased simultaneously.

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