cally contracting frog sartorius muscle. *J. Muscle Res. Cell Motil.* 14: 311–324.

Ford, L. E., A. F. Huxley, and R. M. Simmons. 1977. Tension responses to sudden length change in stimulated frog muscle fibres near slack length. *J. Physiol.* 269:441–515.

Huxley, A. F., and R. M. Simmons. 1971. Proposed Mechanism of force generation in striated muscle. *Nature.* 233:533–538.

Huxley, H. E., and W. Brown. 1967. The low angle x-ray diffraction diagram of vertebrate striated muscle and its behaviour during contraction and rigor. *J. Mol. Biol.* 30:384–433.

Huxley, H. E., A. R. Faruqi, M. Kress, J. Bordas, and M. H. J. Koch. 1982. Time-resolved x-ray diffraction studies of the myosin layer line reflections during muscle contraction. *J. Mol. Biol.* 158:637–684.

Huxley, H. E., R. M. Simmons, and A. R. Faruqi. 1989. Time course of spacing change of 143 Å meridional crossbridge reflection during rapid shortening. *Biophys. J.* 55:12a. (Abstr.)

Huxley, H. E., R. M. Simmons, A. R. Faruqi, M. Kress, J. Bordas, and M. H. J. Koch. 1983. Changes in the x-ray reflections from contracting muscle during rapid mechanical transients and their structural implications. *J. Mol. Biol.* 169:469–506.

Irving, M., V. Lombrdi, G. Piazzesi, and M. A. Ferenczi. 1992. Myosin head movements are synchronous with the elementary force-generating process in muscle. *Nature.* 357:156–158.

Martin-Fernandez, M. L., J. Bordas, G. P. Diakun, J. E. Harries, J. Lowy, G. R. Mant, A. Svensson, and E. Towns-Andrews. 1994. Time-resolved x-ray diffraction studies of myosin head movements in live frog sartorius muscle during isometric and isotonic contractions. *J. Muscle Res. Cell Motil.* 15:319–348.

Piazzesi, G., V. Lombardi, M. A. Ferenczi, H. Thirwell, I. Dobbie, and M. Irving. 1994. *Biophys. J.* 68:92s–98s.

Press, W. H., B. P. Flannery, S. A. Teukolsky, and W. T. Vetterling. 1987. Numerical Methods: The Art of Scientific Computing. Cambridge University Press, Cambridge, U.K.

Squire, J. 1981. The Structural Basis of Muscular Contraction. Plenum Press, New York.

DISCUSSION

Session Chairperson: Ivan Rayment
Scribe: David Lawson

RHEA LEVINE: Can you tell me if you have any indication of what percentage of the heads might be in each of the two populations?

JOAN BORDAS: Well, that's the problem. In order to get that number from the diffraction data, one has to know unambiguously the form factor of each population, and that is not known. One can speculate that the axial orientation of both populations is roughly similar during the stretches because they are subjected to similar strain. If that is the case, then one would deduce that they are occupied on a 50% basis. But that is the best one can do.

HUGH HUXLEY: The separation of the centers of the two halves of the A-band is just less than 0.9 μm, and the separation between the two peaks you've seen is a little over 1 μm. Those two are rather close together, and because of the shape of the peak and the way you sample it, I think you could get errors in that direction in the measured separation depending on how you measure the peak position. So I was wondering whether at least part of what you're seeing mightn't be interference between the two halves of the A-band. In that case, one would get extremely complicated effects when you took the tension off and the two halves came slightly closer together and at the same time some heads left actin and started diffracting at the shorter spacing.

BORDAS: There are a number of reasons why we do not believe this is the case. First of all, the distance between the two interfering units would have to be of the order of 10,000 Å to explain the presence of this splitting.

HUXLEY: 1 μm.

BORDAS: 1 μm, right. Now for this to happen, it would obviously be dependent on the sarcomere length, which we haven't observed. We also have noticed that other reflections which arise from these kind of interference effects vanish during contraction. This is because there is a certain amount of axial translation between filaments that makes them vanish. That argues against it also. Why would these particular reflections be preserved? The third reason why we have not considered an interference effect as an explanation is why would these reflections have such different time courses? And why would one of the time courses follow tension so accurately while the other one doesn't? In addition, we see similar effects in the high order reflections. For instance, the 72 shows the same effects. The one important difference, which is probably interesting, is that its total intensity does not change, but the two contributions behave the same way. Also, other higher order reflections appear to do the same thing. Putting all these things together, it would be very difficult to explain the observations on the basis of interference effects. One would expect, at least, to have different effects on different reflections.

HUXLEY: One of those I don't think would give any difficulty—the effective change of sarcomere length—because that won't effect the interference between the 2 halves of the given A-band.

BORDAS: If the interference is across the M-line, that's true. But isn't that a little bit too short?

HUXLEY: Well, that's the one that's 0.9 μm.

BORDAS: What we have measured is about 1000 Å longer than that. So it doesn't fit in.

HUXLEY: If you think of shape of the sampling as the curve you're sampling, you could get a small error in that direction.

BORDAS: I don't believe that we're making an error of 1000 Å.

VINCENZO LOMBARDI: My question is about the two populations. I think it is intrinsic to a conventional cross-bridge model like the Huxley-Simmons model that there is a distribution among the different states of attached cross-bridges, and this distribution depends on the mechanical condition. For instance, Gabriella Piazzesi's model downstairs indicates that there are three states, of which only the first two are populated in isometric conditions. When you deliver a ramp or a step length perturbation, the new distribution will be attained with each state showing its own kinetics. Thus, these states can provide different time courses for the associated structural signal. Have you thought in these terms?

BORDAS: I don't see a problem with that. In fact, the simplest structural interpretation one can give to these two periodicities is something like that. This is difficult to explain quickly, but I'll try. If you would consider an actin filament surrounded by three myosins and you ask the heads to go to the nearest actin, they will do so in a fairly non-strained sort of way. You will end up with a repeat that corresponds to about 21 actin subunits. That could be one periodicity, the fourth order of which will be about 14.4 nm. If the other head in each pair then went to a situation where this is strained, it would give you a periodicity of 16 actin subunits, the third order of which will be about 14.6 nm. So yes, it fits in.

LEEPO YU: If that is the case, then you would see splitting in rigor. And in rigor conditions you know for sure that both heads are attached.

BORDAS: My group is probably doing these experiments just at this moment.

MICHAEL REEDY: Joan, you've dealt with Hugh's concern that it might represent interference effects between two different sarcomeres. My question is directed at something a little different. What's the size of the diffracting unit? How homogeneously can these two populations be mixed? Or to what extent do they have to be in separate microcrystalline regions that are uniform, for each periodicity in adjacent areas, in order to satisfy the intensities and behavior you've seen?

BORDAS: I can only give you a minimum size, not the maximum size, because the estimate is ultimately limited by how accurately we can measure the width of the reflection. This is, of course, convoluted with the direct beam spot, and beyond that we cannot measure. On this basis, the actual length of the diffraction can be estimated to be at least 3800 Å, but it could be much longer than that. We do not know how much longer.