In this work, we made no attempt to measure the amount of cholesterol effectively incorporated in the membrane of GH3 cells. However, as the C/P ratio of purified fractions of GH3 plasma membranes is relatively low (approximately 0.3)[19], liposome preparations having a C/P ratio of 0.5 or more would produce a cholesterol enrichment[12].

Basic electrophysiological properties of GH3 cells were partly restored by treating the cholesterol-enriched cells with empty liposomes (fig.). 20 min–1 h after the addition of empty liposomes mean membrane potential rose again to -41.7 mV± 2.1 ($n=22$); mean membrane resistance to 83 ± 10.2 MΩ, and 63% of the cells tested ($n=22$); were excitable. Amplitude and shape of action potentials were also restored by the treatment, which presumably depleted membrane cholesterol from the overloaded cell membranes (fig.). These observations suggest that enrichment and depletion of cholesterol were achieved through specific exchanges rather than through a process of fusion. Moreover they show that the effect of cholesterol on the membrane of GH3 cells is reversible.

It has been shown that altering membrane cholesterol content affects the microviscosity of the membrane[12] and thereby modifies the mobility of membrane enzymes and transport channels. The results reported here suggest that membrane ionic channels associated with the electrophysiological properties of GH3 cells are also affected by changes of membrane cholesterol content. Increasing the cholesterol content of membranes depolarizes GH3 cells and affects the shape of action potentials by prolonging the repolarization phase. These observations suggest that an experimental enrichment of membrane cholesterol of GH3 cells affects potassium channels. Similar actions have been reported for tetraethylammonium. (TEA) and 4-aminopyridine (4 AP)[5,14], 4 AP blocks potassium channels in a variety of excitable cells but enhances the influx of Ca^{2+} during depolarization[15] and thereby stimulates the release of secretory products[14,16]. The release of PRL by GH3 cells has been shown to be stimulated by 4 AP[14]. By prolonging action potentials and depolarizing GH3 cells, cholesterol-enriched liposomes also enhance PRL release by these cells independently of the presence of secretagogues. The table shows that incubation of GH3 cells with cholesterol enriched liposomes ($C/P=0.5$) is associated with a 60% increase of PRL release whereas empty liposomes are ineffective. This increased release of PRL, though less pronounced than the release induced by TRH, is significant.

Various physiological and pathological conditions may affect the microviscosity of biological membranes[17,18]. Such changes, by interfering with the properties of ionic channels of pituitary cells, may induce significant alterations of hormonal release.

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Relationship between light diffraction intensity and tension development in frog skeletal muscle

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Summary. Laser diffraction intensity decrease in active muscles precedes tension development at sarcomere lengths below 2.76 μm, but not at greater lengths. This suggests that the time lag is caused by random sarcomere shortenings inside each myofibril.

Recently we have shown that activation of skeletal muscle fibers results in a large decrease in the diffraction intensity of first order line and this is due to myofibrillar misalignment[1]. A theoretical approach to this problem has shown that light diffraction studies may be used to investigate the myofibrillar organization of the active fiber[2,3]. On the other hand, many investigators (using the X-ray diffraction method[4–6], measurements of sarcomere shortening and stiffness[7,8], and the light scattering method[9]) have recently found that the crossbridges move out in less time than is necessary for tension to develop in fibers at approximately the slack length of the sarcomere. These findings allow us to suggest that during fiber activation, myofibrillar alignment becomes disordered faster than the process of tension development. The time lag between diffraction intensity decrease and tension development can be considered a consequence of slight and random sarcomere shortenings inside each myofibril.

Materials and methods. Single fibers from the semitendinosus of *Rana nigromaculata* were dissected in Ringer's solution composed of (mM): NaCl(115), KCl(2.5), Na_2HPO_4 (2.15), NaH_2PO_4(0.85), $CaCl_2$(1.8) adjusted to pH 7.0 and maintained at 2 °C. The intact fiber was mounted in a cooled chamber (2–4 °C) fitted with a glass opening through which the laser beam (NEC, Model GLG 5350) was directed. When mechanically skinned fibers were used, the fibers were prepared from *Rana catesbeiana* as described elsewhere[1]. In the present work, the fiber sarcolem-

ma was completely removed for a distance of 4–7 mm and the non-skinned area was dissected out. Fiber activation was accomplished by electrical stimulation (30 Hz, 0.5 msec duration for 0.5 sec) of an intact single fiber along its entire length with a pair of Pt wires, and by applying activating solution containing pCa 5.49 to a skinned fiber. The details of the experimental system including detection of diffraction intensity have been described previously[1,3]. Isometric tension was recorded simultaneously with the optical change using an isometric transducer (Nihon Koden, TB-216T).

Results and discussion. In figure 1, a decrease in the diffraction intensity of first order line is shown together with the tension recording in intact fibers stimulated tetanically. The decrease proceeded more rapidly than the tension development at sarcomere lengths of 2.46 and 2.76 μm, although the onset of intensity decrease was simultaneous with the onset of tension development. The half-maximum of intensity decrease was reached 8.8 ± 1.3 msec (N = 11, mean ± SEM) before that of tension development over a range of sarcomere length from 2.23 to 2.76 μm. The same phenomenon was observed in skinned fibers activated by Ca^{2+} (fig. 2, a–c). The result was just the same as that in intact fibers; that is, the intensity decrease preceded the tension development (time lag = 1.5 sec). These data imply that the myofibrillar misalignment precedes the tension development, based on our previous suggestion that myofibrillar misalignment inside an active fiber results in a major decrease in the diffraction intensity[1,3]. The myofibrillar misalignment upon activation is caused by slight and random sarcomere shortenings in each myofibril and/or bundle(s), because the intensity decrease and the sarcomere shortening could not be observed at long sarcomere lengths beyond thick-thin filament overlap[1]. It appears that sarcomeres shorten at the expense of elastic elements and that this shortening is complete before the tension reaches its final value[7]. Thus, stretching of the fiber leads to a maximum stretch of elastic elements and may result in the disappearance of the time lag between diffraction intensity decrease and tension development. To investigate this possibility we activated stretched fibers. In intact or skinned fibers at sarcomere lengths above 3.12 μm, the rate of decrease in the diffraction intensity was completely synchronized with the time course of tension development (fig. 1 d and 2 d). The time lag between diffraction intensity change and tension development was less in a stimulated single fiber at a sarcomere length of 2.93 μm than when the length was below 2.76 μm. The synchronization of diffraction intensity and tension curves in stretched fibers is due to deceleration of optical signal change, because the maximum velocity of tension development was approximately the same at all conditions (data in fig. 1 are obtained from 4 different intact fibers). The different rates of intensity and tension in fibers at shorter sarcomere lengths may be considered a consequence of the sarcomere misalignment in each myofibril taking place at random and the sarcomere alignment of short fiber apparently being loose, as opposed to the alignment of the stretched ones. This is supported by our unpublished data in which an intensity increase of more than 10-fold was obtained by passive stretching of the fiber sarcomere length from 2.4 to 3.6 μm in a mechanically skinned fiber. Baskin et al.[10] have reported a similar result with intact single fibers. The light intensity remained approximately constant for 7.5 sec after the fiber had started to relax at all sarcomere lengths observed (fig. 2). Optical measurements of the first order line width show a considerable broadening for a brief period after relaxation, indicating that a certain degree of heterogenity exists inside the fiber at this time.

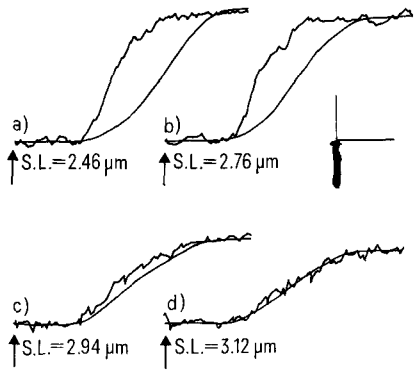

Figure 1. Simultaneous recordings of diffraction intensity and tension development in 4 different intact single fibers at various lengths of sarcomere. Each fiber was stimulated tetanically starting at the arrow. Upward deflections indicate decrease in intensity and increase in tension. To permit accurate comparison of time course, both curves were superimposed. Sarcomere length (SL) is that before stimulation. Note that the intensity decrease precedes the tension development in a, b and c, but not in d. It is meaningless to compare the maximum velocity of tension development between traces, because data are obtained on different fibers. Calibrations; vertical bar, 20 mg, 6% of the light diffraction intensity at initial sarcomere length (I_0), horizontal bar, 10 msec.

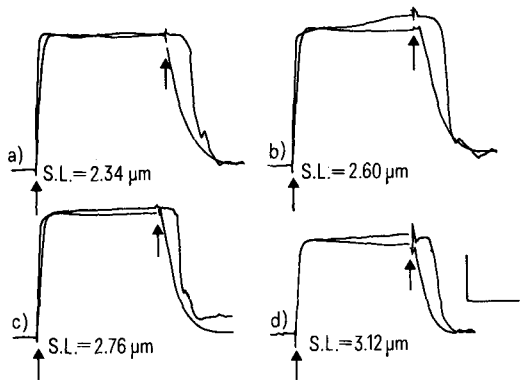

Figure 2. Diffraction intensity and tension development in skinned fiber activated by pCa 5.49. The fiber was activated at the first arrow and relaxed at the 2nd arrow. The maximum velocity of tension development can be compared between traces, because data are obtained in 1 fiber. See figure 1 for other information. Calibrations; vertical bar, 40 mg, 30% of I_0, horizontal bar, 30 sec.

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