decrease in labeling when tissues of mice treated with cycloheximide were used. Recently, an attempt was made to determine the distribution of ornithine decarboxylase by the use of conjugates of DFMO with rhodamine or biotin (*8*). This method depends on the specific binding of such derivatives to ornithine decarboxylase. This was not demonstrated unequivocally and appears unlikely since ornithine decarboxylase has little affinity for substrate analogs with additions to the amino groups (*9*). The method also depends on the maintenance of ornithine decarboxylase activity in sections fixed with glutaraldehyde and formaldehyde or on the passage of the DFMO conjugates across the cell membrane. The present use of labeled DFMO avoids these difficulties.

The histological autoradiographs obtained in the present study suggest that ornithine decarboxylase in the proximal tubule cells occurs predominantly in the cytoplasm. This is in agreement with many studies in which enzyme activities were assayed in extracts from various subcellular fractions, but there have been a few reports of a nuclear location for ornithine decarboxylase [see (*10*)]. Our results do not rule out the possibility that a small proportion of the enzyme is present in organelles such as the nucleus. Studies with [^{3}H]DFMO might help to resolve this question.

The induction of a number of proteins in the mouse kidney in response to androgens is well established and it is known that androgen administration results in hypertrophy of the proximal tubule cells and an increase in the content of microsomal and lysosomal β-glucuronidase (*11*). The present results indicate that the induced renal ornithine decarboxylase, a cytoplasmic enzyme, is also present in the proximal tubules. These results strengthen the association between the activity of this enzyme and cellular hypertrophy. Androgens produce significant increases in RNA synthesis in the mouse kidney, and a number of messenger RNA's that are specifically induced by androgens have been isolated although not all of the gene products are identified (*11*). The mouse kidney should, therefore, provide a valuable system in which to study the induction of ornithine decarboxylase.

Our results contrast with a report that rat kidney ornithine decarboxylase is located predominantly in the medullary region as determined by dissection of the kidney and measurement of the activity in extracts prepared from homogenates of the cortex and medulla (*12*). Ornithine decarboxylase is not androgen dependent in the rat kidney and rat kidneys do not show the marked hypertrophy of the proximal tubules observed in the mouse.

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Muscular Contraction: Kinetics of Crossbridge Attachment Studied by High-Frequency Stiffness Measurements

Abstract. *Instantaneous stiffness of frog skeletal muscle, an indication of the proportion of attached crossbridges, was determined during the tetanus rise and after a step length change imposed during the tetanus plateau. During the onset of contraction as well as after a step, the ratio of stiffness to force differed from that determined during the tetanus plateau. The data after a step are predicted by the Huxley-Simmons model of muscular contraction, but the results during the rise suggest that a long-lived state may exist between crossbridge attachment and force generation.*

It is widely accepted that the contractile force of a striated muscle fiber develops because of interactions between actin and myosin filaments in each half-sarcomere. These interactions also produce an increase in fiber stiffness, which is thought to be directly proportional to the number of attachments (crossbridges) between actin and myosin filaments (*1, 2*). To estimate the number of crossbridges attached during the rise of tetanic force and during the phase of quick recovery from a step length change imposed on the tetanus plateau, we determined the moment-to-moment relation between stiffness and force. Our data indicate that the ratio of stiffness to force is higher during the quick recovery phase than during the plateau, which is predicted by the theory Huxley and Simmons (*1*) proposed to account for the molecular mechanism of contraction. However, we observed that the rise in stiffness preceded the development of force during the onset of contraction. This may be due to the existence of a long-lived crossbridge state between attachment and force generation.

To determine stiffness we applied small sinusoidal length changes (amplitude, 0.02 to 0.05 percent of fiber length; frequency range, 1 to 9 kHz) to one end of a fiber and recorded the corresponding changes in force at the other end. Stiffness was calculated as a ratio between the force and length sinusoids. In order to compare our data with some of the data in the literature (*3*), we also used step length changes to determine stiffness. The forces recorded at the end of step length changes of various amplitudes were plotted against the sizes of the length changes. We thus obtained "T_1 curves" (*1*), which allowed us to estimate the amount of compliance in the end attachments of our preparations by comparing our results to those obtained by Ford *et al.* (*3*), who used a servo system to control the length of a fiber segment between two markers. The in-

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fluence of the end regions of a fiber is eliminated by their method. We used the amount of instantaneous shortening necessary to just discharge the force developed by a fiber (that is, "y_0") as an indication of the compliance in a preparation. We determined y_0 from T_1 curves (for instance, Fig. 1) as well as from the ratio between force and length sinusoids.

The T_1 curves in Fig. 1 were obtained on the tetanus rise and plateau with a single fiber from the lumbricalis" digiti IV muscle of a frog (*4*). Extrapolation of the linear portion of the curve at the plateau yields an intercept (y_0) of −5.3 nm per half-sarcomere for zero force on the length axis. This value is similar to that of about −5.7 nm per half-sarcomere estimated at a similar temperature (3.7°C) from Ford *et al.* (*3*). For a release of 8.1 nm per half-sarcomere the actual value of relative force was 0.02 times the maximum tetanic force (P_0), which is comparable to the value of $0.035P_0$ at 7.5 nm per half-sarcomere estimated from Ford *et al.* (*3*). These results indicate that the influence of end attachments was small enough that the elastic properties of the preparation could be attributed mostly to the muscle fiber itself.

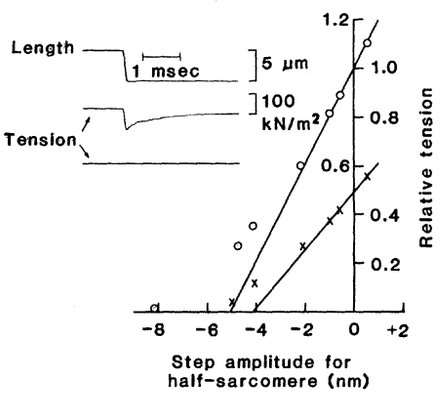

Fig. 1. The T_1 curves (*4*) obtained during (X) the tetanus rise and (○) the plateau of force development with a lumbricalis" digiti IV fiber (9.vii.81). Length steps during the tetanus rise were performed 60 msec after the start of stimulation. (Inset) Length step (upper trace) and force response (lower trace) corresponding to the third release point on the linear portion of the T_1 curve at the plateau; the bottom line indicates zero force. The fiber was stimulated by 0.5-msec pulses applied transversely at 15 Hz and three times rheobasic strength. The experiment was conducted at 4°C. Sarcomere length was 2.25 μm (measured by laser diffraction). Overall length of the fiber at this sarcomere length was 2.53 mm, and maximum tetanic tension was 292 kN/m^2. Length amplitudes are expressed in nanometers per half-sarcomere on the assumption that length changes were uniformly and entirely distributed along the fiber. Fibers were dissected clean to the ends of the cells to make the points at which tendons attached clearly visible. Fiber diameter (maximum and minimum) was measured and used to calculate tension, assuming an elliptical fiber cross section. Force and length changes were recorded on a digital oscilloscope (Nicolet Explorer). The minimum time per point was 5 μsec. Length changes were produced with a low-inertia moving coil system (*9*), and steps could be completed in less than 130 μsec. Force measurements were made with a capacitance force transducer (*10*) with a natural frequency of 50 kHz and a compliance of 0.05 μm/mN. The use of short fibers (lumbricalis) made it possible to use fast steps and high frequencies to measure stiffness.

The value of y_0 during the tetanic plateau was also calculated from measurements obtained with sinusoidal length changes. For the fiber of Fig. 1, y_0 at 1, 4, 7, and 9 kHz was 6.1, 4.6, 4.3, and 4.2 nm per half-sarcomere, respectively. Hence stiffness increased with the frequency of oscillation. The increase was greatest in the range 1 to 4 kHz and became less pronounced in the range 4 to 9 kHz. The relatively small stiffness at low frequencies of oscillation was due to the quick recovery of the force (*1*). This is supported by the observations that force preceded length by about 50 μsec (phase angle, 18°) at a frequency of 1 kHz, while no phase shift was detectable in the range 4 to 9 kHz.

Stiffness during recovery from a quick release was reduced in comparison to stiffness during the tetanus plateau. This is in accordance with previous work on longer fibers (*5, 6*). This reduction in stiffness was much smaller than the reduction in force. For example, after a release of 3.7 nm per half-sarcomere, force was reduced to $0.41P_0$ in the experiment of Fig. 2. When force had recovered to $0.5P_0$, stiffness was already at 0.8 of its maximum tetanic value. Stretches caused a small increase in stiffness, but once again considerably smaller than the increase in force.

According to the theory of Huxley and Simmons (*1*), stiffness should remain constant during the quick recovery, and consequently the ratio of stiffness to force after a release should be higher than that determined during the tetanus plateau. The ratio of stiffness to force in our experiments was higher than the plateau value during the quick recovery from a release, although a fall in stiffness was usually observed. The reduction in stiffness found during the recovery from a step release could indicate detachment of crossbridges, but could also be due to tendons whose compliance does not obey Hooke's law. In order to decide between these possibilities, we performed a slow release (900-μsec step time) with length oscillations superimposed. In this way, it was possible to measure stiffness during the release as well as during the recovery. The effects of tendon compliance were canceled by making stiffness measurements at the same force during the recovery and the release. For the fiber described in Fig. 1, the stiffness measured at $0.7P_0$ during the recovery was slightly less (4 percent) than during the release. In order to explain this result in terms of crossbridge behavior, one must suppose that detachment of crossbridges is brought about by the release. Although a quick release may cause detachment of crossbridges, it seems unlikely that reattachment occurs in such a way that the same relations between stiffness and force are maintained during the quick recovery and during the release. Hence the presence of a non-Hookean tendon compliance seems a more likely explanation for the apparent reduction in stiffness.

Stiffness was also determined during the tetanus rise (Fig. 2). Throughout this period stiffness was higher than predicted by a constant ratio of stiffness to force. The T_1 curves obtained from measurements during the tetanus rise (Fig. 1) also showed that stiffness was higher than expected for a constant ratio of stiffness to force. The rise in stiffness during the onset of contraction preceded the rise in force by about 15 msec. This result was obtained with frequencies

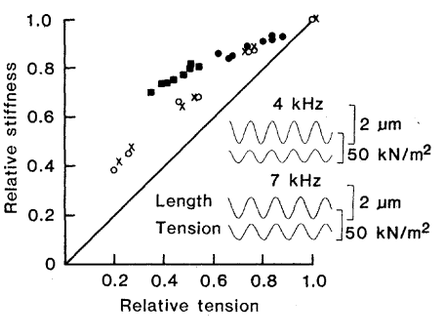

Fig. 2. Relation between relative stiffness and relative tension during tetanus rise [(X) 4 kHz; (○) 7 kHz] and after step release [(■) large; (●) small, 7 kHz] for the same fiber as in Fig. 1. Points during the tetanus rise were measured at 38.0, 42.5, 58.0, 62.5, 88.0, and 92.5 msec after the start of stimulation. Stiffness was measured from the mean peak-to-peak amplitude of four consecutive peaks for force and length. Points during quick recovery were obtained from releases of two different amplitudes [(■) 9.34 nm and (●) 3.63 nm per half-sarcomere]. The first 4 msec after completion of the step were analyzed with the ratio of peak-to-peak amplitudes for force and length in each individual cycle. Analysis of quick recovery from the large release (■) was begun after force had recovered to $0.35P_0$. (Inset) Length (upper traces) and force (lower traces) at 4 and 7 kHz. These records correspond to the points obtained at about $0.5P_0$ on the tetanus rise.

from 1 to 9 kHz although the effect was greater at frequencies higher than 1 kHz.

Although the compliance of the tendons could influence the stiffness measured on the tetanus rise, especially in this very short preparation, we find it unlikely that tendon compliance could account for our data (*7*). The lead of stiffness over force during the tetanus rise can be explained by assuming there is a relatively long-lived crossbridge state between attachment and force generation. Such a state was proposed by H. E. Huxley (*8*) on the basis of the observation that the equatorial x-ray diffraction pattern from contracting frog muscle changes more rapidly than force during the tetanus rise.

To summarize, the fall in force after a step release is accompanied by a much smaller fall in stiffness. Most of this fall in stiffness is due to tendon compliance. This is consistent with observations reported by others (*5*, *6*). However, the fact that stiffness increased more rapidly than force during the tetanus rise suggests that crossbridge attachment might be followed by a significant delay before force develops.

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4. Isolated single muscle fibers were dissected from lumbricalis" digiti IV muscles of *Rana temporaria*. Aluminum clips were attached to the tendons at each end to reduce tendon compliance. The fibers were then transferred to a small chamber and suspended between a length step generator (*9*) and a force transducer (*10*). The amount of compliance in a preparation was estimated by performing rapid step length changes and plotting the force at the end of the step against the step amplitude to obtain T_1 curves (*1*). The amount of instantaneous shortening sufficient to just discharge isometric force (y_0) was determined by extrapolating the linear portion of the T_1 curve (for instance, Fig. 1). In some experiments on single fibers from tibialis anterior muscles, the stiffness measured by sinusoidal length changes had higher apparent values than expected and there was a considerable phase lag of force relative to length. Thus fibers behaved like a uniform elastic rod in which a natural mode of vibration was excited by length oscillations. Since tibialis anterior fibers were longer than those from lumbricalis" digiti IV muscles (5.6 to 7.3 mm compared to 1.5 to 2.6 mm), their resonance frequency was lower. The resonance frequency of the fiber used for Fig. 1, estimated from the transmission time, was about 40 kHz during the tetanus plateau. This is in good agreement with the theoretical value obtained by assuming a fiber behaves as a uniform elastic rod and viscous forces are negligible [M. Schoenberg, J. B. Wells, R. J. Podolsky, *J. Gen. Physiol.* **64**, 623 (1974)].
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7. (i) A different interpretation is possible if we assume that our data may be influenced by the elastic properties of the tendons. If there were a constant ratio of stiffness to force during the tetanus rise, and if the amount of instantaneous shortening necessary to just discharge the force developed by the crossbridges was assumed to be constant at about 4 nm per half-sarcomere (*3*), it should be possible to estimate tendon compliance on the tetanus rise and on the plateau from the observed value of y_0. Such measurements imply that there was higher tendon stiffness on the rise than at the plateau, which is unlikely. Our results could also be explained by assuming that a large portion of the fiber compliance resides in the filaments and the Z line. However, the compliance of the filaments and Z line is likely to be only about 10 percent of the total fiber compliance (*2*); this is too small to account for our results. (ii) Another alternative is that the low level of fiber stiffness during the tetanus rise reduced the resonance frequency of the fiber, causing significant amplification of the force oscillations. This may have some basis at very low levels of stiffness. However, the relative stiffnesses at 4 and 7 kHz during the tetanus rise were not significantly different and did not show detectable phase shift. Furthermore, calculations on the assumption that a fiber behaved as an undamped elastic rod—the worst possible case—indicated that such an effect is not large enough to account for the observed deviation from a constant ratio of stiffness to force. (iii) The presence of some degree of shortening against tendon compliance could be another explanation for our findings. According to previous data [A. F. Huxley and R. M. Simmons, *Cold Spring Harbor Symp. Quant. Biol.* **37**, 669 (1973); F. J. Julian and M. R. Sollins, *J. Gen. Physiol.* **66**, 287 (1975)] and the model of A. F. Huxley [*Prog. Biophys. Biophys. Chem.* **7**, 255 (1957)], shortening would tend to increase the ratio of stiffness to force. However, a rough calculation shows that an improbably large amount of shortening at the expense of tendons would have to occur during the tetanus rise. The first point on the tetanus rise in Fig. 2, which was measured with 4-kHz oscillations, has a ratio of 1.9. According to the Huxley model, such a ratio is only attained at a shortening velocity of 2.2 mm/sec (0.37 times the maximum velocity, V_{max}). At the second point measured with 4 kHz the ratio is consistent with a velocity of 1.8 mm/sec (0.30 V_{max}). The time elapsed between these measurements was 4.5 msec. Taking the worst case—the slowest velocity—as the average shortening velocity during this period, then the tendons should have been extended by 8.1 μm as force increased from 0.22 to $0.27P_0$. However, the T_1 curve for this fiber indicates that the extension of the total fiber compliance from 0.22 to $0.27P_0$ was less than 1.8 μm. Consequently, such a degree of tendon extension seems impossible.
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Loss of Retinal X-Cells in Cats with Neonatal or Adult Visual Cortex Damage

Abstract. *Recordings were made from single retinal ganglion cell somas in cats whose visual cortical areas 17 and 18 were damaged on the day of birth or in adulthood. Neonatal lesions produced a 78 percent loss of X-cells in the retina, while lesions made in adulthood produced a 22 percent loss. Y-cells and W-cells were unaffected. This retinal abnormality needs to be considered when interpreting studies of behavioral deficits and neural mechanisms of recovery after damage to the visual cortex.*

Damage to visual cortical areas of the brain in adult mammals (including humans) results in severe deficits in visual abilities, although some behavioral recovery does occur (*1*). If similar brain damage is incurred neonatally, the resultant behavioral disabilities can be much less severe; indeed, the animals can perform normally on many tasks when mature (*1*). In correspondence with these behavioral results, anatomical and physiological studies indicate that anomalous neural connections can develop in the central visual pathways after neonatal, but not adult, brain damage (*2*). This suggests that functional compensation by the central nervous system may underlie the superior recovery of behavior after neonatal lesions. But to fully understand the neural basis of both the initial deficits and the subsequent recovery, it is necessary to know the nature of the inputs to the system.

Anatomical studies indicate that the retina is abnormal after visual cortical damage. Marked transneuronal retrograde degeneration of retinal ganglion cells follows cortical lesions in neonatal cats and monkeys, and less severe degeneration follows lesions in adult monkeys and humans (*3–7*). Analysis of soma sizes and central projections of the remaining ganglion cells in the affected retinas led to the suggestion that the degenerated ganglion cells are primarily of the X-cell (*8*) functional class (*4*, *5*, *7*). However, the physiological properties of the retina after visual cortical damage have never, to our knowledge, been studied in any species. We therefore sought to determine the functional properties of retinal ganglion cells in adult cats given visual cortical lesions as neonates or as adults. We found that W- and Y-type ganglion cells are present in normal numbers and have normal response properties. However, a marked loss of X-type ganglion cells follows neonatal lesions and a smaller loss follows adult damage.

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