seawater, were then assayed for glucanase activity. Enzyme release from the particles is followed by its excretion into the surrounding seawater. The timing of the enzyme release parallels that of changes in light-scattering which accompany breakdown of cortical granules (*14*). This correspondence is consistent with the common identity of cortical granules with the large enzyme-containing particles.

The enzyme-containing particles are unstable in sucrose, and hence this compound cannot substitute for NaCl in the homogenization media. Since sucrose is also a known parthenogenetic agent causing lysis of the cortical granules (*4, 15, 16*), the instability of the particles in sucrose also lends support to the identification of the large glucanase-containing granules with the cortical granules. Additional evidence is that agents removing the cortical granules from intact eggs also result in removal of β-1,3-glucanase from the egg. Thus, incubation of unfertilized eggs in isotonic solutions of nonelectrolytes, such as 1*M* urea, discharges and dissolves the cortical granule contents (*4, 16*). If unfertilized eggs are incubated for 1 minute in 1*M* urea (*p*H 8.0), 45 percent of the glucanase is removed from the eggs and can be recovered in the urea supernatant.

Although final proof must await electron microscopic studies, the above evidence is consistent with the hypothesis that β-1,3-glucanase is associated with large particles which are the cortical granules. Undoubtedly, other enzymes, such as an acid phosphatase (*17*), are also present in the cortical granules. Utilization of membraneless eggs to collect and characterize enzymes and other constituents released at fertilization (*18*) may further clarify the chemical basis of the fertilization reactions.

The function of β-1,3-glucanase is the most intriguing aspect of this study. Behavior of the enzyme suggests that its activity is not solely concerned with fertilization, since 60 percent of the enzyme remains within cytoplasmic particles after insemination. Our studies show this remaining enzyme is gradually excreted from the egg during the cleavage and blastula stages and that the bulk of enzyme is gone by gastrulation.

Consideration of the enzymes' substrate specificity, its latent nature within particles, and its release from the egg suggest that it is involved with metabolism of extracellular or cell surface glycoproteins during both the fertilization reactions and the cleavage period. Since the solubilized glucanase is not retained within the egg or upon the egg surface, the critical action of this enzyme must occur coincidentally with its excretion. One model for this critical action is the hydrolytic unmasking of reactive groups, as in the fibrinogen reactions (*19*). Alternatively, if the enzyme acts as a transferase (rather than as a hydrolase) with its natural substrate (*20*), the critical action could be the rapid interchange of polysaccharide side chains and resultant modification of glycoproteins.

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Laser Diffraction Studies on Single Skeletal Muscle Fibers

Abstract. *Sarcomere movements during isometric tetanic contractions were resolved to 50 angstroms by diffraction techniques. After a latent period that followed the first stimulus, all sarcomeres shortened simultaneously and uniformly. Oscillations in length and tension in synchrony with the stimuli occurred during an incomplete tetanus. However, no oscillations in length were detected during the plateau of a fused tetanus.*

While it is widely accepted that contraction of the muscle cell involves a sliding movement of interdigitating filaments within the sarcomere, little is known about the kinetics of the mechanism underlying the propulsion of the filaments and the maintenance of steady-state tension. According to the sliding-filament model (*1*), contraction occurs through summated short-range forces acting between multiple sites along the A (myosin) and I (actin) filaments. Individual sites are assumed to go through repeated cycles of activity and each cycle contributes to the relative movement of the I filament or, during isometric conditions, to the maintenance of tension. Accordingly, the plateau tension of a tetanic contraction reflects an average number of force-producing sites brought into action from moment to moment at the sarcomere length considered.

Larson *et al.* (*2*), with laser diffraction techniques on whole muscle, have reported that small (1.0 to 1.7 percent of rest length) oscillatory changes in the length of the sarcomeres occur at a frequency unrelated to the rate of stimulation during the plateau of a smooth tetanus. This suggests, in terms of the sliding-filament model, that during an isometric contraction, the number of tension-producing links between the A and I filaments undergoes periodic fluctuations with instant-to-instant readjustment of the sarcomere length. Since no fluctuations in tension occur,

these oscillatory changes in length must be out of phase with each other along the length of the preparation so that the overall muscle length remains constant. Because of the complicated distribution of sarcomere lengths in whole muscle and the difficulty in synchronizing the mechanical output of the various fibers, one cannot draw definite conclusions about the behavior of the individual cells from the diffraction pattern obtained. As the question of oscillations within the sarcomere is relevant to an understanding of the molecular basis of muscle contraction, we have investigated this problem in isolated single muscle fibers. Our results show that the sarcomere length remains constant during the plateau of an isometric tetanus.

Single fibers were dissected from the dorsal head of the semitendinosus muscle of *Rana pipiens* and mounted (*3*) horizontally between a tension transducer (RCA 5734) and a lever in a temperature-controlled bath (1 to 2°C) of Ringer solution (NaCl, 115.5 m*M*; KCl, 2.0 m*M*; $CaCl_2$, 1.8 m*M*; NaH_2PO_4–NA_2HPO_4, 2.0 m*M*).

The resting sarcomere length of the fiber, as measured optically (*3*), was set to a value between 2.5 and 2.7 μ. Stimulation was effected by passing current through alternate anodes and cathodes spaced at 2-mm intervals along the length of the fiber; all pairs of electrodes produced a supramaximal stimulus. The tension output of the fiber was displayed on a Tektronix 502A oscilloscope and recorded photographically. The sarcomere length throughout the complete time course of the tension development was measured from the diffraction pattern produced by passing a laser beam (*4*) (632.8 nm; beam diameter, 2 mm) perpendicularly to the longitudinal axis of the fiber. Three orders of diffraction lines symmetrically spaced about the zero-order reference were obtained. Sarcomere spacing relates to the diffraction pattern according to the formula:

$$d \sin \theta = n \lambda$$

where d denotes the sarcomere length, θ the angular separation of the diffraction lines from the zero-order reference, λ the wavelength of the incident light, and n the order of the diffraction line. A slit was placed across the center of the diffraction pattern on the screen so that only the zero-order reference and one first-order line were retained for measurement. Movements of the first-order beam relative to the zero-order reference beam were recorded from the screen onto continuously moving film (Kodak Tri-X, 100 mm/sec). Deflections of the first-order trace could be read with an accuracy of 0.2 percent which corresponds to a change in sarcomere length of 50 Å in the range of sarcomere lengths used here.

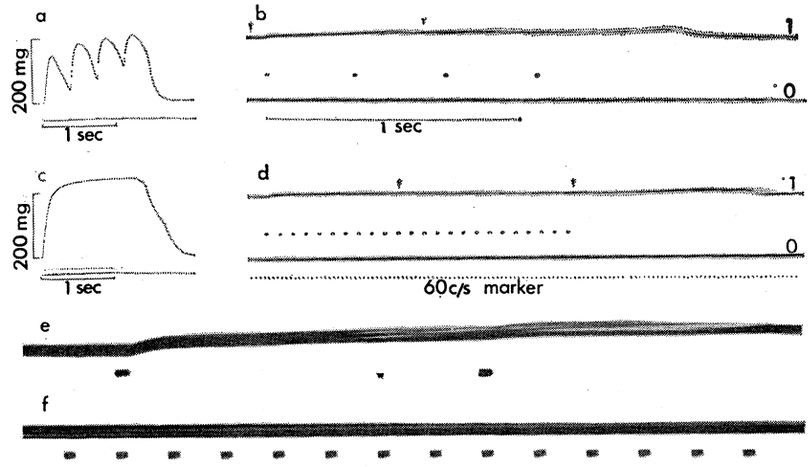

Fig. 1. Recordings of tension (a, c) and zero- to first-order diffraction pattern (b, d) during tetanic contraction of a single muscle fiber of the frog. (a, b, and e) Partially fused tetanus, stimulus intervals 357 msec; (e) a segment of the first-order line of trace (b) lying between the arrows magnified four times. There is a stepwise increase in deflection of the first-order beam in synchrony with stimuli. (c, d, f) Completely fused tetanus, stimulus intervals 49 msec; (f) is a portion of trace (d) lying between the arrows magnified four times. Here no oscillatory changes of either width of deflection of first-order diffraction beam are detectable. Markers below first-order line are stimulation signals. Sarcomere spacing at rest was 2.65 μ.

Recordings of tension and zero- to first-order diffraction patterns during isometric tetani of a single fiber are shown in Fig. 1. After a latent period following the first stimulus, there is a simultaneous onset of tension development and sarcomere shortening. Oscillatory movements of both tension and first-order beam are clearly synchronized with the stimuli during an incomplete tetanus (Fig. 1, a, b, and e). The amplitude of the length oscillations decreases with each successive stimulus. This reflects the increasing stiffness of the series elastic element at the higher levels of tension (*5*).

No fluctuations of either the first-order line width or zero- to first-order line spacing are detectable during the plateau of a fused isometric tetanus (Fig. 1, c, d, and f). Results similar to those described in Fig. 1, d and f, were obtained if diffraction measurements were made at other points along the length of the fiber. This finding emphasizes that all sarcomeres within the fiber contribute uniformly to the tension development at the sarcomere lengths studied (*3*).

Our results do not correspond to those obtained from the whole sartorius muscle of the frog (*2*, *6*). In these, oscillations of the diffraction pattern occurred during the steady state of isometric tension. We have no ready explanation for such results; however, they clearly do not reflect the behavior of a single, functionally intact fiber. From our results it is evident that during the plateau of a tetanic contraction no synchronous length changes of the sarcomeres occur along the length of the fiber. This implies, in terms of the sliding-filament model, that the number of active links between the A and I filaments within a cross section of the fiber does not undergo synchronous fluctuations under steady-state tension. However, our findings do not preclude small asynchronous "to and fro" movements of the A and I filaments, even though the average number of links between the filaments remains constant. This question cannot be adequately resolved from our present data.

An apparent dissociation of length and tension recordings during the relaxation phase appears in Fig. 1, a, b, c, and d, where an increase in the deflection of the first-order beam (indicating a further shortening of the sarcomeres) occurs when the tension output of the fiber is progressively declining. This

phenomenon probably reflects differences in duration of the active state in different regions along the length of the fiber with the result that portions of the fiber having the longest active state, shorten at the expense of those parts in which the capacity to produce tension declined earlier (*7*).

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Hibernation Induced in Ground Squirrels by Blood Transfusion

Abstract. *Natural mammalian hibernation was continuously maintained under laboratory conditions throughout spring and summer seasons in a colony of thirteen-lined ground squirrels by serial transfusional passage of blood from hibernating animals to active animals. This procedure successfully produced hibernation in animals until late summer, at which time naturally occurring (spontaneous) hibernations occurred in the colony, thus terminating the experiment.*

Mammals which hibernate, whether in field or laboratory, do not ordinarily do so in midsummer months (*1*). There is some variation between species as the bat and the hamster. Hamsters are capable of hibernation, on occasion, under cold, dark, quiet conditions. Ground squirrels and marmots, on the contrary, very seldom hibernate in the midsummer under any conditions. Hibernation was induced in our laboratory animals in the summer, triggered by the transfusion of hibernation blood.

We maintained a colony of approximately 20 thirteen-lined ground squirrels (*Citellus tridecemlineatus*) in a 7°C, dark, quiet room (cold room). None of these animals hibernated between early March and 7 August except the experimental animals described herein.

During the winter of 1967 to 1968, all of the animals in our colony had hibernated. By 6 March 1968, all had returned to an active state except one animal, which continued to hibernate. This donor was in a state of deep hibernation with a low body temperature and very slow respiration. Without arousing this cold animal, we opened the abdominal cavity and drew 3 ml of blood from the dorsal aorta. An anticoagulant was not used in this procedure. Shortly after this exsanguination the animal died (Fig. 1). The blood was kept cold and 1 ml was injected into each of three animals from a 23°C room (warm room) within a few minutes from the time of withdrawal from the hibernator. Blood was introduced directly into the saphenous vein in two cases, and intraperitoneally in the third case. The injected animals were then placed into the cold room. There was no evidence of anaphylactic shock or other adverse reaction to the transfused blood. Three nontransfused animals from the warm room were placed in the cold room as controls, and they remained there through the summer months without going into hibernation. Both animals that had received blood intravenously began to hibernate after 48 hours (Fig. 1). Thereafter they followed a typical hibernating pattern: going into and out of hibernation for varying lengths of time, from a few days to several weeks, until mid-June. Additionally, they displayed other of the characteristics of true hibernation, including the typical head-down balled-up position, lowered body temperature, and greatly decreased respiration. We later obtained blood in the manner described above from these two animals when they were hibernating, and transfused their blood into three more animals from the warm room, that also hibernated when placed in the cold room (Fig. 1). Two animals from the warm room were transfused in a similar way with blood from an active animal and then placed into the cold room as further controls; these did not hibernate. In July, blood of two of the three hibernating animals was transfused into five more active ground squirrels from the warm room, all of which have now hibernated (13 August 1968, Fig. 1). Our colony now consisted of 24 animals, 14 in the cold room and 10 in the warm room. On 7 August two of the control animals (transfused with blood from an active animal) hibernated. Shortly thereafter, other animals in the cold room that had not been transfused began to hibernate. Since the late summer or fall hibernating season had obviously begun, the experiment (valid only as a test for true hibernation in midsummer) came to a close. No data obtained beyond this date could be regarded as significant.

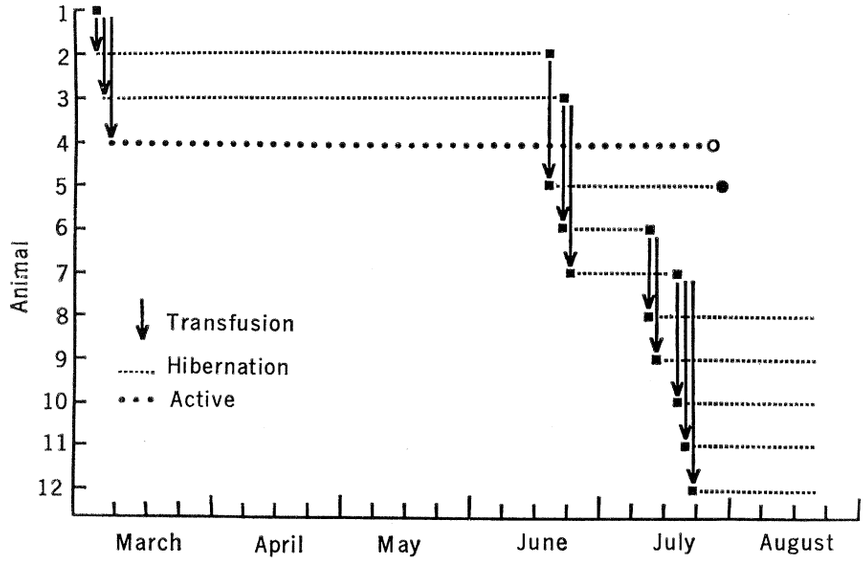

Fig. 1. Transfusions accomplished in spring and summer season (1968) resulting in hibernation of 13-lined ground squirrels (*Citellus tridecemlineatus*). Solid square, hibernating donor; open circle, death (not in hibernation); solid circle, death (in hibernation).