panic caused by thirst makes people wander to an almost certain death within hours. A modest but dependable source of water may prevent these tragedies.

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Sarcolemma: Transmitter of Active Tension in Frog Skeletal Muscle

Abstract. *Maximum tetanic tension developed by frog muscle fibers was measured before and after they had been injured at one end so as to break myoplasmic continuity but to leave the sarcolemma-tube connection to the tendon. The lateral mechanical coupling between myoplasm and sarcolemma is adequate to bear and transmit the maximum active tension developed during stimulation.*

All current models of the structure of striated muscle, such as the sliding-filament hypothesis (*1*) or the somewhat older hypothesis of a folding polypeptide chain, have in common the assumption that the contractile machinery is in the myofibril and that the myofibril transmits the tension developed by it to the tendons. We have thought that force developed by the myofibrils may be transmitted laterally to the sarcolemma and thence to the tendons; we have, therefore, measured the tension developed before and after breaking the myoplasmic column by injury.

Bundles of two or three fibers were isolated from the semitendenosus muscle of the frog *Rana pipiens*. One end of each bundle was cleaned of connective tissue so that, as far as possible, only sarcolemma made the connection to the tendon. Except for the use of No. 26 hypodermic needles as dissecting knives, the techniques were the same as described in 1940 (*2*). The fibers were mounted in a bath of frog Ringer solution kept at 18°C or at room temperature. Tensions were measured with a strain gauge and changes in length with a rack-and-pinion device. Maximum isometric tetanic tension was determined by adjusting the length of the bundle; stimulation was by square-wave pulses, 50 per second and of 1-msec duration. Each bundle was then placed on a microscope slide, stretched slightly, and held in place by tucking the tendons under rubber bands. The end that had been cleaned of connective tissue was injured by gentle pressure with a thin glass rod. Such pressure at first causes strong local contracture; after 10 to 30 minutes a localized clot of myoplasm forms and retracts, forming a retraction clot and leaving only the sarcolemma tube connecting the uninjured part of the fiber and the tendon. When the break in the myoplasmic column was complete in all fibers, the bundle was remounted and maximum tetanic tension was redetermined. The stimulus voltage was doubled. The muscle length at which maximum tension could be developed was greater after injury than before.

The injured fibers sometimes remained excitable for several hours. After injury, seven bundles had active tensions, varying from 30 to 100 percent of the original tension; four single-fiber preparations developed 70 to 80 percent. Experience has shown that an injured fiber is an unstable and failing preparation, so that the variation in results is not surprising, but large tensions were transmitted around a complete myoplasmic break.

Figure 1 shows two single-fiber preparations that were preserved in 50-percent glycerol for 1 day after the experiment. Their appearance is essentially the same as that of living fibers at this magnification.

The one bundle that gave as much tension after injury as before consisted of three fibers, each about 55 μ in diameter. Maximum tension developed before and after injury was 264 mg, or about 3.7 kg/cm^2, which is average for fibers of frog muscle.

The apparently empty stretches of sarcolemma tube are filled with fluid. Electron microscopy (*3*) has resolved the sarcolemma into four layers: the outermost consists of unidentified fine filaments, the next is a braidlike layer of collagen filaments, the third resembles matrix of basement membrane, and the innermost is the plasma membrane. Clotting destroys most of the plasma membrane, but the other three components persist in the sarcolemma tube; tensile strength of the tube is presumably due to the collagen layer.

If the thickness of the sarcolemma is taken as 0.1 μ (*4*), the sarcolemma tubes of the fibers that developed tensions of 3.7 kg/cm^2 bore a tension of 510 kg/cm^2. This is quite similar to the ultimate tensile strength measured in tendon, which is around 600 kg/cm^2 (*5*). Active tension was proportional to the cross section of the uninjured part

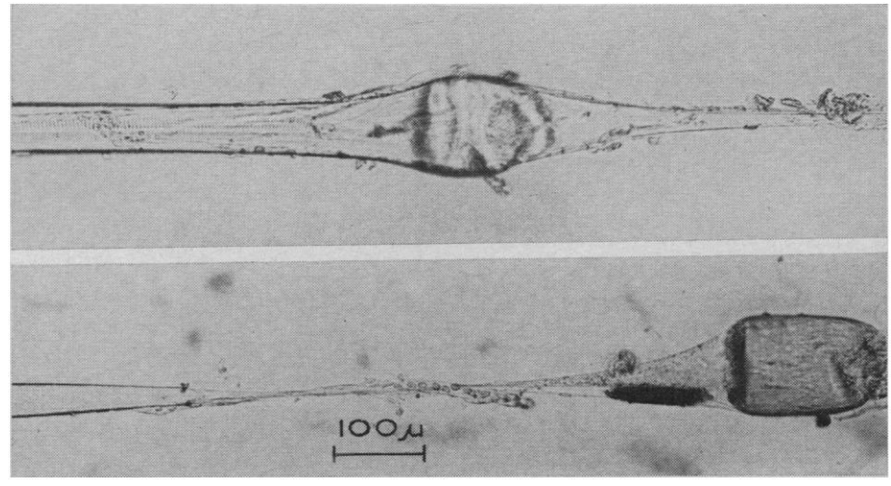

Fig. 1. The injured ends of two single fibers of muscle prepared as described in the text and preserved in 50-percent glycerol. In the larger fiber (top), clear, striated myoplasm at left is in contact with the retraction clot in the middle. The sarcolemma, the dark line at the outer edge of the fiber, continues as sarcolemma tube on the right of the clot and connects to a tendon which is not pictured. In the smaller fiber (bottom) the retraction clot has separated from the myoplasm which appears compressed to a cone. Striations here are irregular and obscured by granules.

of the fiber and was not affected by the loss of contact between the myoplasm and the retraction clot that occurred in the fibers of small diameter. The part of the fiber adjacent to the injury lost excitability.

Some fibers were tetanized while the injured ends were being observed by microscope. Stimulation speeded up coagulation in the large fibers so that a series of retraction clots formed. In the smaller fibers the cones retained their shape during stimulation, and no new clots formed for a considerable time. This observation may be pertinent to the problem of the nature of the mechanical coupling between myoplasm and sarcolemma. The only organelle of the cell known to connect to the sarcolemma is a delicate transverse network of tubules at the level of the Z membrane in each sarcomere (T-tubule system) (*6*). If the myofibrils pull against such a network, one would expect to see a concave surface at the injured end. The cone suggests that a discarded model of muscle, consisting of a viscous gel enclosed in an elastic membrane, with tension transferred by short-range molecular forces, may have some validity (*4*).

Similar experiments incorporating injury were performed in 1951 (*6*); the decrease in active tension reported was probably due to loss of excitability.

In 1940 (*2*) we showed that the relation between length and passive tension in single muscle fibers was approximately the same for both the sarcolemma tube and the intact fiber. The injury experiments just described show that the tube can also transmit active tension; this, we think, occurs not only in the case of a complete myoplasmic break. Many diseases and injuries cause patchy pathological changes in muscle with the result that parts of a single fiber may be normal and parts abnormal. Such fibers from muscle that has been injured by section of one tendon (tenotomy) are often able to develop normal isometric tension. It is probable that tension developed in a normal part can be transmitted to the tendons by the sarcolemma, thus bypassing the damaged section.

In studies of normal muscle, this bypass must be considered in any analysis of the correlation of muscle length and active tension. It is an interesting possibility that the low metabolic cost of negative work (work done by a muscle while it is being stretched) may be due to the sarcolemma bypass rather than to a reversal of chemical reactions.

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Iodination in Relation to Thyroglobulin Maturation and Subunit Aggregation

Abstract. *Noniodinated subunits of thyroglobulin can aggregate, but iodination of the aggregate is required for its stabilization (maturation). Rat-thyroid slices incorporate amino acids into subunits, but cannot form mature thyroglobulin from the newly synthesized subunits. This defect leads to an accumulation of 16S and 12S proteins, although the preexisting thyroglobulin is 19S. Accumulation of 16S and 12S proteins can be produced in rat thyroids by the administration to the animals of a thiocarbamide derivative, methimazole. Upon withdrawal of methimazole, iodination of the 16S and 12S proteins proceeds, and 19S protein appears.*

The iodination of thyroglobulin-like proteins in thyroid slices occurs after completion of the polypeptide backbone (*1–5*), but it is not known whether the presumed subunit precursors (3–8*S* and 12*S*) of thyroglobulin (19*S*) are normally iodinated before aggregation and whether iodination is essential for the aggregation. There probably exists an anatomical, as well as a temporal, separation of iodination and aggregation (*2, 6, 7*). In lamb-thyroid slices after incubation with a labeled amino acid or with ^{125}I, there are three classes of thyroglobulin molecules, each with slightly different physical properties. Iodine-labeled thyroglobulin (18.5*S*) is intermediate in rate of ultracentrifugal sedimentation and in ease of dissociation to its half-molecule (12*S*) when compared with amino acid–labeled thyroglobulin (17.5–18*S*) and with unlabeled, preformed thyroglobulin (19*S*) of the lamb-thyroid slice (*2, 6, 7*). These properties may reflect the relative biologic maturity of the three kinds of thyroglobulin. Iodination appears not to be necessary for subunit aggregation, since amino acid labeling of thyroglobulin occurs in lamb-thyroid slices despite complete inhibition of the incorporation of iodide into organic form by propylthiouracil (*1, 2*).

However, when thyroid slices are incubated for very brief periods with radioactive iodide, the 3–8*S* and 12*S* proteins are more highly labeled than the 19*S* material (*2, 4, 7*). The proportion of radioactivity associated with the subunit proteins decreases with continued incubation. It is not known whether these iodine-labeled smaller units represent breakdown products, derived from an especially labile fraction of thyroglobulin, or whether they are subunits which are iodinated as such and which may or may not undergo aggregation to form thyroglobulin.

In this report we present data which support the concept that iodination, while not essential for aggregation, is required for thyroglobulin maturation.

Rat-thyroid slices incorporate radioactive amino acid primarily into three proteins, all of which sediment more slowly than thyroglobulin (Fig. 1) (*8*). As has been shown for lamb- (*2, 7*) and for human- and calf-thyroid slices (*9*), radioactive amino acid is incorporated into 3–8*S* and 12*S* proteins before label appears in more rapidly sedimenting particles. However, unlike the situation in these other species in which label appears in 17.5–18*S* particles after about a 20-minute lag, in the rat radioactivity accumulates to a large extent in a 16–16.8*S* particle and continues