used for studying Mie scattering from oriented irregular particles. In addition, our observations of levitation in complex light intensity distributions, such as in Fig. 2d, as well as interesting cases of levitation of small drops in the irregularities of the light beam occurring just above splattered fallen drops lying on the cell floor, indicate that levitation does not require an extreme uniformity of geometry. Finally, concerning ice crystals, we believe that fairly regular crystals such as prisms *should* levitate and that more complex shapes *may* levitate. Possibly an electric field would orient the more platelike crystals and thus aid in levitation.

Levitation permits various drop interactions to be observed. Thus, if two levitated drops (Fig. 2b) have opposite charges, we can force them closer and closer together by applying an external field until they finally coalesce. The fused drop remains levitated at a new height with the combined mass and charge. We have also directly observed drop-drop collisions. Often a levitated drop is struck from above by a heavier drop that is drawn into the beam as it falls. Alternatively, a levitated drop can be hit from below by lighter drops that wander into the lower regions of the beam where they are drawn in, are driven upward through the beam focus, and then encounter the levitated drop (see Fig. 2a). In these encounters we have often observed drop coalescence when the partners have opposite charges and misses when the drops have like charges. Drops can grow by a factor of 4 or 5 in diameter by successive collisions.

The simple experiments described here indicate the potential of the technique for studying the important problems of droplet growth by accretion in Langmuir type collisions with the use of cloud size droplets. In more sophisticated experiments prepared target and incident drops of known size and charge, each initially held in its own optical trap, could be used. They could subsequently be placed in the same beam and be brought together at varying speeds, with an electric field or light being used as the driving mechanism. The parameters of the collision, the particle trajectories, and the detailed fluid dynamics of the drop coalescence could be observed with high-speed movie cameras viewing from different angles. Finally we have made observations on the question of the coalescence efficiency of drops making physical contact. Occasionally we observed two drops roughly equal in size come side by side in the beam and seemingly touch for seconds prior to coalescence. This result should be checked with the use of movie cameras, as suggested above.

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14 November 1974

Human Skeletal Muscle: Properties of the "Chemically Skinned" Fiber

Abstract. *A "skinning" procedure is described for irreversibly disrupting the sarcolemmal membrane of human skeletal muscle and allowing calcium and other diffusible solutes (such as adenosine triphosphate) access to the myofilament space. Single skinned fibers give isometric tensions of about 1.5 kilograms per square centimeter when exposed to ionized calcium even after 1 to 2 weeks of storage at 5°C. For up to 5 days the preparation will sequester and, under appropriate conditions (anion substitution, caffeine addition, or magnesium withdrawal), release calcium. The regulation of intracellular calcium distribution and the calcium-induced activation of the contractile proteins are discussed and related to the morphology of human fibers and to similar processes occurring in other muscle preparations.*

The sarcolemmal membrane of human skeletal muscle fibers (*1*) becomes permeable to high molecular weight ions such as adenosine triphosphate (ATP) after 1 to 2 hours of immersion in a solution lacking ionized calcium (pCa > 9). The solution (R) contains 5 to 10 m*M* ethylene glycol-bis(aminoethylether)-*N,N'*-tetraacetic acid (EGTA), 170 m*M* potassium propionate (KPr), 2 m*M* magnesium acetate, 2 m*M* Na_2ATP, and 5 m*M* imidizole and has a pH of 7.00. The permeability changes that result from storing the sarcolemma in solution R occur at 5°C and apparently are due to physical disruption of the membrane, since only fragments of this organelle can be seen in electron micrographs of the stored fibers (*2*). The extensive disruption of the sarcolemma undoubtedly accounts for both the effectiveness and the irreversibility of the "skinning" procedure. Once the sarcolemma is sufficiently disrupted to allow the chemical constituents of the bathing medium access to the myofilament space, the fibers are referred to as having been chemically skinned.

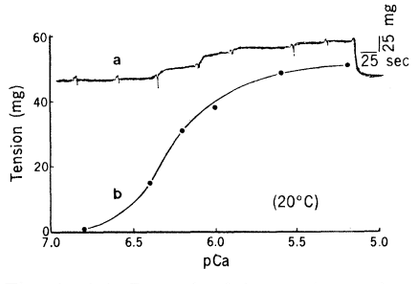

Fig. 1. (a) Record of isometric tensions given by a single skinned fiber exposed to a series of increasing Ca ion concentrations. The Ca was buffered with a total of 5 m*M* EGTA, and the final pCa was calculated as described in (*13*). The concentrations of the other bathing solution components are as in solution R (see text). (b) A tension threshold for this fiber appears at about pCa 6.7 and the maximum tension (P_o) at about pCa 5.2.

The sarcolemma of human skeletal muscle immersed in an EGTA-based solution is much more labile than the sarcolemmas of other skinned fiber preparations such as frog cardiac muscle (*3*) and frog (*4*) and crayfish (*5*) skeletal muscle. Frog and crayfish skeletal muscle exposed to an EGTA-based solution require mechanical skinning for the sarcolemma to be disrupted sufficiently to allow the chemical constituents of the bath access to the myofilament space. Frog cardiac muscle, however, becomes permeable to high molecular weight ions when exposed to EDTA (ethylenediaminetetraacetic acid), but the membrane regains its semipermeability when reexposed to millimolar concentrations of ionized Ca (*3*).

Once skinned, a 2- to 4-mm length of single muscle fiber was placed in a temperature controlled (20°C), vig-

orously stirred bath (*5*) and secured at either end by Lucite clamps, one of which was attached to a strain gauge mounted on a micromanipulator. The sarcomere length was then adjusted to be in the range of 2.9 to 3.1 μm, which was found to give a maximal isometric tension for these skinned fibers of about 1.5 kg/cm^2.

The relation of the ionized Ca concentration in the bath to the steady state tension is shown in Fig. 1b for a fiber with a diameter of 95 μm. The Ca was buffered with EGTA (total EGTA = 5 m*M*). The tension recording from which the plot of tension against *p*Ca was determined is shown in Fig. 1a. The first detectable tension displacement occurred at *p*Ca 6.4, although this value varied among fibers from different biopsies, and a range from *p*Ca 6.8 to *p*Ca 6.0 has been noted. In Fig. 1 the tension reached a near-maximum value (P_0) at *p*Ca 5.2, although again, a variation among biopsies has been noted with a range from *p*Ca 5.8 to *p*Ca 5.0. The skinned human skeletal muscle fiber has a Ca sensitivity that is similar to that of various preparations from a variety of muscle types, such as skinned crayfish skeletal muscle (*6*) and glycerinated psoas and vascular smooth muscle from rabbit (*7*). As in other preparations (*6*) the Ca sensitivity varies with the substrate (MgATP) concentration, although more data are needed to describe this relationship.

The response of the chemically skinned human fiber to treatments that are believed to mobilize Ca from the sarcoplasmic reticulum (SR) is similar to the responses given by other skinned skeletal muscle fibers (*8*). It is interesting that these similarities occur despite the scarcity of longitudinal SR elements in human skeletal muscle (*9*). The human SR, instead, is composed mainly of large cisternae that form triadic structures with the transverse tubular system along the A-I junction (*9*).

The processes involved in sequestering and releasing Ca within the skinned fiber were studied by analyzing the development of isometric tension under the following conditions: fibers were exposed first to a solution (W) which contained the same components and had the same ionic strength as the skinning solution but which lacked EGTA. While in W if the fibers were exposed to ionized Ca at concentrations ranging from 10 to 100 μM they would attain P_0, although it might take many minutes before either the onset or the complete development of that tension. The delay in the onset and relatively slow development of the tension has been studied in other preparations, and has been ascribed most recently to the combined effects of Ca sequestration by the SR and Ca binding to nontroponin sites (X) within the fiber matrix (*8, 10*). After the fibers were exposed to an unbuffered Ca solution, but while they were still at rest, a transient tension could be induced by (i) replacing KPr with KCl, (ii) adding caffeine (1 to 40 m*M*), or (iii) reducing the concentration of free Mg while keeping the substrate MgATP concentration constant (*11*) (Fig. 2, upper tracings). The transient tensions that are thought to result from sequential release and uptake of Ca by the SR (*8, 11*) were abolished by treating the fiber with the nonionic detergent Brij-58 (Fig. 2, lower tracings). Brij-58 destroys the ability of the SR to retain Ca (*10, 12*). An observation which indicates to us that X sites exist in human fibers is that adding Mg to a Brij-treated fiber caused a step increase in tension, and removing it caused a step decrease (Fig. 2, lower tracings). The relation between *p*Ca and tension was not affected by the concentration of free Mg required for this response, and the response was not observed in the presence of 10- to 50-μM free EGTA. It is concluded, therefore, that Mg displaces Ca bound to X sites in human skeletal muscle. This binding of Ca to X must be considered when interpreting the kinetics and amplitudes of tension during activation and relaxation.

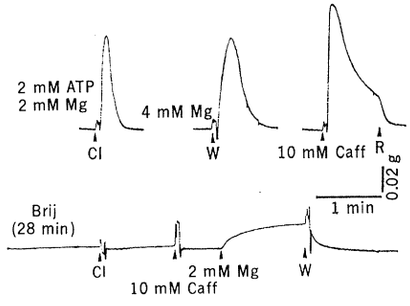

Fig. 2. (Upper tracings) Transient tension elicited by three different methods from a single skinned fiber primed with Ca in solution W (preexposure to Ca not shown; see text for solution components). At the first arrow propionate was replaced by chloride, which induced a transient tension. After the fiber was reprimed with Ca in a solution containing 4 m*M* excess Mg, the excess Mg was removed, which induced another transient tension. The fiber was then reprimed with Ca and caffeine (*Caff*) was added, which induced a third transient tension. (Lower tracings) The same fiber, after exposure to 0.5 percent Brij-58 for 28 minutes, no longer gave a tension response when chloride was substituted for propionate or when caffeine was added to the bathing solution. Addition of Mg, however, caused an increase and its subsequent removal a decrease in tension.

Thus the intracellular processes involved with tension development and Ca regulation can be studied in single human fibers. The efficacy of the chemical skinning procedure coupled with the tissue's long-term viability makes it possible, with very small quantities of tissue, to analyze these processes in diseased as well as normal muscle.

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