paration with non-ionic detergents that destroy the sarcolemma[4].

We therefore suggest that a third criterion for determining whether a preparation is chemically skinned should be added to the two used by Miller of sensitivity of the muscle to Ca and lack of resting potential: that a preparation is chemically skinned if tension responses affected by MgATP are consistent with tension responses of mechanically skinned or non-ionic detergent-treated preparations. Because the behaviour of EGTA-treated mammalian skeletal muscle fibres[4–7] meets this criterion, there can be no doubt that they come under the definition of a chemically skinned preparation[5].

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MILLER REPLIES—Reuben and Wood have defined a critical test of whether a muscle fibre may be considered skinned by treatment with EGTA or EDTA. This test provides an admirable basis against which to check all types of fibre, in addition to the skeletal fibres on which their own work has been carried out. However, their criteria differ markedly from those of Winegrad[1] which are still generally accepted (for review see ref. 2) and applied[3], and which I contend to be ambiguous[4]. Indeed, additional criteria, such as the acceleration of the rate of tension development at higher Ca-buffer levels[1], are also met by unskinned preparations[5] where extracellular exchange is the limiting process.

My own investigations on frog heart (on ventricular muscle, with which the technique was established[1], and auricle) demonstrated that (1) the method fails to produce the effects claimed for it, and (2) there are alternative explanations for the observed phenomena[4]. Subsequent work with the EGTA/EDTA method has, therefore, been founded on an incorrect interpretation and its validity ought to be re-examined in that light. Where additional evidence of skinning, preferably along the lines indicated by Reuben and Wood, has not been provided, the conclusions remain potentially open to criticism. This is not to say that all such preparations will prove, like frog heart, not to have been skinned, but that evidence broadly based on Winegrad's original criteria alone is not compelling. Wood *et al.*[6] had reported ultrastructural evidence of skinning by EGTA in skeletal muscle and the detailed results published more recently[7] amply confirm their evidence of skinning. However, the feature of the method responsible for the skinning effect remains obscure as prolonged exposure to EGTA (80 mM) in itself does not lead to membrane disruption[8,9].

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MILLER[1] has challenged the notion that the permeability of the sarcolemma of cardiac muscle can be markedly enhanced by treating the tissue with either EDTA[2] or EGTA[3,4]. He has claimed that the two major criteria generally used to infer the existence of the high permeability state, that is, low transmembrane potential and increase in force at very low Ca concentrations, can be produced without an accompanying increase in membrane permeability, and he has concluded that only microdissection of the sarcolemma[5] can produce a cardiac cell in which one can be sure that the membrane barrier to the movement of ions has been removed and the sarcoplasmic reticulum and mitochondria retained.

Although Miller's conclusions may apply to his own studies on EDTA-treated frog ventricle[1], it is less clear that they apply to my EDTA-treated frog ventricular preparation, as I found that the resting potential was not restored for many minutes after the re-introduction of normal Ringer's bathing solution (compare ref. 1, Fig. 1 with ref. 2, Table 1). In addition, in my preparation at *p*Ca 5.4, tension was almost 90% of maximum whereas in Miller's it was less than 50% of maximum in spite of the same Na concentration. Miller's results do highlight the difficulty of producing a hyperpermeable state in frog heart with EDTA and focus on the reason for developing a better preparation.

Miller's conclusions are incorrect for hyperpermeable cardiac fibres that can be produced in rat ventricular muscle by treatment with EGTA as there are at least six different properties of this preparation that favour the existence of a high membrane permeability to small ions and molecules.

(1) The amount of force and the rate at which the force develops at any given concentration of Ca are very stable and reproducible[4].

(2) A stable rigor state is rapidly initiated when ATP has been removed from the bath and rapidly reversed when ATP is restored. This occurs in spite of the maintenance of the concentrations of Na and K and the elevation of the concentration of imidazole to keep the ionic strength constant. In the absence of ATP, the addition of phosphocreatine rapidly relaxes the rigor state only if ADP is present[4]. The relaxation therefore does not depend on a change in the concentration of Na.

(3) The membrane potential of individual cells was stable at −8 mV for long periods when all the KCl had been replaced with NaCl as well as in the presence of Ca concentrations as high as 0.03 mM. According to Miller and Mörchen[6], reduction of Ca to 0.02 mM prolongs the action potential in frog only 20–200%, with little change in resting potential. In rat ventricle Garnier *et al.*[8] found the duration of the action potential prolonged to only a few seconds by Ca-free solutions. Because in our study the same cell was impaled for several minutes, the depolarised state could not be due to a long plateau of an action potential.

(4) The Ca-induced Ca releases from the sarcoplasmic reticulum that were produced by lowering the concentration of EGTA to 30 μM and raising Ca to sub-threshold concentration were very rapidly terminated by raising the EGTA concentration to 3 mM[4]. The very large increase in tension produced by caffeine-induced Ca release from the sarcoplasmic reticulum in 0.03 mM EGTA was totally blocked by 3 mM EGTA[4].

(5) Although treatment of the EGTA-treated cells with detergent for 30 min destroys the function of the cell membranes, it does not alter either the rate of development of force at similar levels of tension or the maximum Ca-activated force.

(6) Possibly most important in refuting Miller's argument is the fact that the amount of force generated at a given concentration of Ca is not altered by changing the Na concentration in the bathing solution. The Na concentration was varied from 10 mM to 40 mM over a range of *p*Ca of 9.0–4.5 with no change in the force generated at any concentration of Ca. These results indicate that Ca, ATP and EGTA all cross the membrane very rapidly and that gradients of K and Cl cannot be maintained across the membrane.

We have described the EGTA-treated cell as hyperpermeable because it is not the same as a mechanically skinned cell. The cell membrane remains, and it is still a diffusion barrier for large molecules such as creatine phosphokinase and glutamate oxaloacetate transaminase. This is not a limitation, but rather an advantage, as it allows the direct probing of the contractile proteins with Ca in a preparation that more closely resembles the intact cell, and regulatory mechanisms that are lost in the mechanically skinned fibre are retained in