the EGTA-treated hyperpermeable cells.

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MILLER REPLIES—To answer Winegrad's conclusion that frog heart is rendered 'hyperpermeable' by EDTA/EGTA treatment: the discrepancy in time scale for the restoration of normal resting potential between his study and my own[2,3] can have several causes. First, my preparations are considerably smaller, are rapidly superfused and display tension responses within seconds[2,4] rather than minutes[1] of solution changes. Slow extracellular exchange will contribute to delayed resting potential recovery. Second, Winegrad reported on the recovery of the resting potential after prolonged exposure to the full 'skinning' medium. It seems most likely that the intracellular ionic levels could alter in these circumstances (high $[K]_0$, low $[Na]_0$, depolarisation, low $[Ca]_0$) and contribute to the slow redevelopment of resting potential in normal Ringer's solution. Third, Winegrad's results were obtained with "an exploring microelectrode" (ref. 1, p. 79). This makes a reliable measurement of time course difficult as substantial repolarisation must occur to give a clear indication of a successful impalement with the microelectrode.

The differences in tension levels at *p*Ca 5.4 noted by Winegrad are hardly indicative of qualitative differences between preparations. He has reported elsewhere that tension developed at this (or any other) *p*Ca is strongly influenced by the immediate history of the preparation (for example, see ref. 5, Fig. 4). Quantitative differences among frog species[4] and between auricle and ventricle (ref. 2 and my unpublished observation) do occur. However, in thin, rapidly superfused ventricle trabeculae exposed to a regimen identical to that of Winegrad, rapid ($t_{1/2} <$ 5 s), complete relaxation always occurs when $[Na^+]_0$ is raised to 120 mM and $[K^+]_0$ reduced to 3 mM (*p*Ca 5.4). This finding signifies more than the "highlighting of the difficulty of producing a hyperpermeable state".

I will discuss the six properties which Winegrad considers "favour the existence of a high membrane permeability to small ions and molecules" in rat ventricular muscle. Properties (1) and (3) have been dealt with already (see above and refs 2, 3). With respect to property (2), removal of ATP as reported in Winegrad's publications had seemed to be associated with Na removal (ATP present as Na_2ATP[1,5–7]). In experiments of the type that I previously reported[2], removal of Na_2ATP evokes a submaximal transient tension development (direct comparison is difficult as Winegrad's records are not calibrated in absolute or relative tension). After this the preparation becomes insensitive to *p*Ca steps[2,3]. The tension transient is blocked if NaCl (10 mM) is included in the ATP-free medium. These effects are also seen in frog trabeculae exposed to EGTA treatment overnight. Clearly, ATP removal does not produce rigor tension in frog. These comments are not necessarily appropriate to Winegrad's experiments in the rat. However, two points can be made.

First, tension development in these conditions is not necessarily rigor tension. Stiffness measurements would form one potentially definitive line of evidence for or against the occurrence of rigor and hence the hyperpermeable state. Second, if the rat cells are not 'hyperpermeable' then the effects of ATP removal, and ADP and phosphocreatine addition[6], would be extracellular. Extracellular ATP was responsible for qualitative changes in the behaviour of the frog preparations[2]. The concentrations of ATP ($MgATP^{2-}$, ATP^{4-}?) involved await detailed analysis. ADP and phosphocreatine have yet to be investigated, but they could act in tandem outside the cell. However, as Winegrad has noted[7], leakage of creatine kinase is increasingly used as an index of myocardial damage. This raises the possibility that a low, but significant, level of extracellular ATP is produced by both nonspecific sites and by creatine kinase leakage from damaged cells. Certainly, this type of experiment is potentially capable of differentiating between skinned and non-skinned cells (see also Reuben and Wood's comments above).

To discuss property (4), even with rapid superfusion of very small preparations, $\geq$0.1 mM EGTA (in nominally Ca-free media) is required to prevent tension development in Na-poor solutions[4]. Tension oscillations in these poorly Ca-buffered conditions cannot readily distinguish between Ca-induced Ca-release phenomena in intact and skinned cells as they are triggered by local $[Ca^{2+}]$ fluctuations in both cases: extracellular $[Ca^{2+}]$ near the sarcolemma for intact cells and $[Ca^{2+}]$ adjacent to the sarcoplasmic reticulum for skinned cells. Extracellular $[Ca^{2+}]$ steps produce Ca release in K-depolarised frog heart[8] and oscillatory responses have been reported[9].

Referring to property (5), the comparability of both the rate of tension development and the maximum force in EGTA- and detergent-treated cells is not a definitive property. Ca-exchange would be limited by the gross morphology of the preparations whether the cell membranes are 'hyperpermeable' or not (see also first paragraph). However, the Ca-sensitivity of detergent-treated preparations differs markedly from that of EGTA-treated cells, half-maximum tension occurring more than 1 *p*Ca unit lower in the latter[6,7]. Winegrad attributes this to a modifying effect of fragments of the sarcolemma persisting after EGTA treatment and subsequently removed by detergents. This idea contrasts markedly with the criteria suggested by Reuben and Wood (see above). Similarly, Winegrad's interpretation of the effects of 5 mM theophylline as occurring through phosphodiesterase inhibition[7] rather than Ca release from sarcoplasmic reticulum (like caffeine and other methylxanthines[10,11]) seems unduly selective.

Considering property (6), the sharp sensitivity of EDTA/EGTA-treated frog heart to $[Na^+]$ provides a clear-cut distinction from that of skinned fibres. In frog, the curve relating tension to $[Na^+]$ is shifted more than one order of magnitude towards higher $[Na^+]_0$ by Winegrad's media compared with 'simple' Na-poor solutions (see ref. 2, Fig. 3 and ref. 4, Fig. 4). As these effects have not been quantified for the mammal one cannot assume that any particular $[Na^+]$ will produce relaxation, but levels higher than 40 mM are worth trying. (Note: $K_{Na}^{app} \cdot [Na]_0^4$ in Fig. 3, ref. 3, is given as

$$\frac{1}{K_{Ca}[Ca]^2} + \frac{K_{Na}}{K_{Ca}[Ca]^2} \cdot [Na]^4$$

which does not significantly affect the shape of the curve.)

The failure of the EGTA 'skinning' technique has been demonstrated for frog heart, and a plausible mechanism presented to account for the findings which had proven misleading[2]. The criteria for successful skinning as previously applied to frog are, therefore, inadequate. As a result, a definition of skinning in other tissues is not at all straightforward, but the ideas presented by Reuben and Wood should form a useful starting point.

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