Two properties of the calcium-sensitizing factor can be distinguished. The first is that it can neutralize the inhibitory activity of the inhibitory factor. This property is exhibited in the absence of tropomyosin with those inhibitory-factor preparations that do not require added tropomyosin for activity. In these circumstances removal of traces of Ca^{2+} from the system by the addition of ethanedioxybis(ethylamine)tetra-acetate do not restore inhibition, as occurs with complete relaxing protein systems. On subsequent addition of tropomyosin the Ca^{2+} -sensitivity of the Mg^{2+} -stimulated ATPase is restored and the whole relaxing protein system is reconstituted.

Under conditions of preparation involving prolonged exposure to ethanedioxybis(ethylamine)-tetra-acetate a modified form of calcium-sensitizing factor can be prepared that, although it neutralizes the inhibitory factor, no longer restores Ca^{2+} -sensitivity to the system even in the presence of tropomyosin (Schaub *et al.* 1972). It is suggested that the regulation of the neutralizing action of the inhibitory factor by Ca^{2+} is regulated through an allosteric site on the calcium-sensitizing factor.

The role of the 37000-dalton component is not yet clear. As prepared by chromatography in dissociating conditions it does not replace inhibitory factor, calcium-sensitizing factor or tropomyosin in the relaxing protein system. It is a different protein from these last three proteins as judged by its amino acid analysis. Although its amino acid composition is similar to that of the troponin I described by Ebashi *et al.* (1971) of molecular weight 40000, the latter in some reported functions resembles the inhibitory factor. Ebashi *et al.* (1971) claim complete reconstitution of the relaxing protein system with tropomyosin, troponin I and troponin II. Greaser & Gergely (1971), however, report results that suggest that, in addition to tropomyosin, inhibitory factor and calcium-sensitizing factor, a 35000-dalton component, troponin fraction 3 (possibly corresponding to the 37000-dalton component described by Wilkinson *et al.* 1971) is also required. In our hands reconstitution of the relaxing protein system has been obtained in some circumstances with tropomyosin, inhibitory factor and calcium-sensitizing factor alone.

The extreme difficulties experienced in separating the components of the troponin complex cleanly from each other are probably responsible for the uncertainties still remaining in the nature of the relaxing protein system. Thus on the balance of evidence currently available it can be concluded that the troponin complex consists of three proteins, two of which have well-defined activities whereas further work is required to decide on the precise role of the third component of 37000 molecular weight.

- Ebashi, S. (1963). *Nature, Lond.*, **200**, 1010.
 Ebashi, S., Wakabayashi, T. & Ebashi, F. (1971). *J. Biochem., Tokyo*, **69**, 411.
 Greaser, M. L. & Gergely, J. (1971). *Fedn Proc. Fedn Am. Socs exp. Biol.* **30**, 1309 (Abstr.).
 Hartshorne, D. J. & Mueller, H. (1968). *Biochem. biophys. Res. Commun.* **31**, 647.
 Hartshorne, D. J. & Pyun, H. Y. (1971). *Biochim. biophys. Acta*, **229**, 698.
 Schaub, M. C. & Perry, S. V. (1971). *Biochem. J.* **123**, 367.
 Schaub, M. C., Perry, S. V. & Häcker, W. (1972). *Biochem. J.* (in the Press).
 Wilkinson, J. M., Perry, S. V., Cole, H. & Trayer, I. P. (1971). *Biochem. J.* **124**, 55P.

The Calcium Pump in the Sarcoplasmic Reticulum

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The degree of contraction of a muscle cell is controlled by the intracellular concentration of Ca^{2+} . When the concentration of this ion is increased above about $0.1 \mu\text{M}$ by a nervous stimulus the cell changes from a resting to an active state. Full contraction occurs at about $1.0 \mu\text{M}$ - Ca^{2+} . In skeletal muscle the source of the regulatory Ca^{2+} is the internal membrane system, the sarcoplasmic reticulum. During the relaxation process the Ca^{2+} concentration within the myofilaments is lowered once again to below $0.1 \mu\text{M}$ by the action of the ATP-requiring calcium pump of the sarcoplasmic reticulum (Ebashi & Endo, 1968).

The characteristics of this pump system have been extensively investigated since Ebashi & Lipmann (1962) showed its presence in fragmented sarcoplasmic reticulum of homogenized muscle cells. Earlier reports suggested that univalent cations either had no effect (Martonosi & Feretos, 1964) or inhibited Ca^{2+} uptake (Lee, Tanaka & Yu, 1965). On homogenization in sucrose medium without added salts the endogenous K^+ concentration of the microsomal fraction was lowered sufficiently to show that K^+ increased Ca^{2+} uptake about three-fold, the decreasing order of effectiveness being: $\text{K}^+ > \text{Na}^+ > \text{NH}_4^+ > \text{Rb}^+ > \text{Cs}^+ > \text{Li}^+$. Li^+ has only a slight effect. On incubation of the microsomal fraction in the macrocyclic polyether dicyclohexyl-18-crown-6 (Pedersen, 1968), which forms a stable complex with K^+ , the cation lying in the hole of the polyether ring, the rate of Ca^{2+} uptake falls to a low value. The maximum rate of transport was restored when K^+ was added to the medium, indicating an absolute requirement of this ion by the Ca^{2+} -uptake system. Th^+ , which can replace K^+ in the activation of acetyl phosphatase, *p*-nitrophenyl phosphatase (Inturrisi, 1969a) and

$\text{Na}^+ + \text{K}^+$ -stimulated adenosine triphosphatase (Inturrisi, 1969b) of ox brain, and at one-tenth the concentration also stimulates the calcium pump at much lower concentrations than does K^+ .

When Ca^{2+} uptake is taking place at a decreased rate in the absence of K^+ many barbiturates and phenothiazines have a stimulatory effect. Amylo-barbitone is the most active of the barbiturates, Ca^{2+} uptake being increased by about 300% at 2mM concentration. Chlorpromazine is the most potent of the phenothiazines, being active at about one-thousandth the concentration of K^+ (Duggan, 1971). At a K^+ concentration optimum for Ca^{2+} uptake all the barbiturates and phenothiazines decrease Ca^{2+} transport, in agreement with previous reports (Balzer, Makinose & Hasselbach, 1968; Azzone, Azzi, Rossi & Milic, 1966). It is considered that the stabilizing effect of barbiturates and phenothiazines on biological membranes generally is mediated by this mechanism whereby the displacement of Ca^{2+} from membrane binding sites, the basis of membrane labilization, is diminished. Chlorpromazine has been shown to decrease the leakage of L-aspartate aminotransferase (EC 2.6.1.1) from hypoxic heart to the control value (Langslet, 1971).

Rabbit muscle microsomal fraction has been concentrated and separated from mitochondria on a sucrose density gradient in a BXIV zonal rotor and re-run on a shallower sucrose density gradient. Sealed vesicles with a high rate of Ca^{2+} uptake and low associated adenosine triphosphatase activity were obtained. The distributions of NAD nucleosidase (EC 3.2.2.5), NADH-cytochrome c reductase (EC 1.6.2.1), adenosine triphosphatase (EC 3.6.1.4) and AMP deaminase (EC 3.5.4.6) are sufficiently different to show that some sub-fractionation of the muscle microsomal fraction has been achieved by this technique.

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- Azzone, G. F., Azzi, A., Rossi, C. & Milic, G. (1966). *Biochem. Z.* **345**, 322.
 Balzer, H., Makinose, M. & Hasselbach, W. (1968). *Arch. exp. Path. Pharmac.* **259**, 151.
 Duggan, P. F. (1971). *Eur. J. Pharmac.* **13**, 381.
 Ebashi, S. & Endo, M. (1968). *Prog. Biophys. molec. Biol.* **18**, 123.
 Ebashi, S., Endo, M. & Ohtsuki, I. (1969). *Q. Rev. Biophys.* **2**, 351.
 Ebashi, S. & Lipmann, F. (1962). *J. Cell Biol.* **14**, 389.
 Langslet, A. (1971). *Eur. J. Pharmac.* **13**, 292.
 Lee, K. S., Tanaka, K. & Yu, K. S. (1965). *J. Physiol., Lond.*, **179**, 456.
 Martonosi, A. & Feretos, R. (1964). *J. biol. Chem.* **239**, 648.
 Inturrisi, C. E. (1969a). *Biochim. biophys. Acta*, **173**, 567.

- Inturrisi, C. E. (1969b). *Biochim. biophys. Acta*, **178**, 630.
 Pedersen, C. J. (1968). *Fedn Proc. Fedn Am. Socs exp. Biol.* **27**, 1305.

Structural Changes during Muscle Contraction

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There has been general agreement for some time now that contraction in striated muscle is brought about by a process in which actin and myosin filaments are forcibly caused to slide past each other. It is also agreed by many people that the most likely way in which the sliding force is developed is by a cyclical chemical and mechanical interaction taking place at cross-bridges between the two types of filaments.

More recent X-ray evidence (Huxley & Brown, 1967; Huxley, 1968, 1969) has indicated that cross-bridge movement depends essentially on an active change in the effective angle of attachment of the heads of the myosin molecules to the actin monomers in the actin filaments. Other evidence indicates that it is the attachment process itself that is blocked by the regulatory protein system (tropomyosin and troponin) in a relaxed muscle. Electron-microscopic studies (Moore, Huxley & DeRosier, 1970) utilizing the three-dimensional reconstruction technique have analysed the structural details of this attachment in the 'rigor' configuration, which probably corresponds to the end of the working stroke in an actively contracting muscle, and have shown that the bridges are tilted in the expected direction. Further studies by J. Spudich & H. E. Huxley (unpublished work) have applied the same technique to a study of the location of the regulatory proteins in the thin filaments; these results will be described, together with recent X-ray diffraction studies on contracting muscles that show evidence for a substantial structural rearrangement in the thin filaments on activation. The latter may represent a change in position of tropomyosin, making possible the attachment of a myosin head carrying ATP (or its split products), in the configuration corresponding to the beginning of the working stroke, an attachment that is apparently blocked by tropomyosin plus troponin in the relaxed muscle.

- Huxley, H. E. (1968). *J. molec. Biol.* **37**, 507.
 Huxley, H. E. (1969). *Science, N.Y.*, **164**, 1356.
 Huxley, H. E. & Brown, W. (1967). *J. molec. Biol.* **30**, 383.
 Moore, P. B., Huxley, H. E. & DeRosier, D. J. (1970). *J. molec. Biol.* **50**, 279.