Hasselbach W & Makinose M. Die Calciumpumpe der "Erschlaffungsgrana" des Muskels und ihre Abhangigkeit von der ATP-Spaltung. *Biochemische Zeitschrift* 333:518-28, 1961.
[Inst. Physiologie, Max-Planck-Inst. für Medizinische Forschung, Heidelberg, Federal Republic of Germany]

The findings published in this paper demonstrate that the calcium uptake of muscle microsomes is coupled to the activity of a calcium-dependent ATPase and that calcium accumulation results in the formation of steep calcium concentration gradients. [The *SCI*® indicates that this paper has been cited in over 590 publications since 1961.]

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"In the late-1950s, after *in vitro* experiments performed with isolated contractile proteins had furnished conclusive evidence[1] that ATP was the sole energy source of the contractile apparatus, the problem emerged as to how the ATP hydrolysing activity of the contractile proteins in the living muscle was switched on and off during a contraction relaxation cycle. The road was opened by the discovery of Marsh[2] showing that in aqueous muscle extracts a factor was present which could suppress the contraction of isolated contractile proteins and that this effect could be abolished by calcium ions.

"In 1957, we found that the microsomal particles were very similar to the vesicular fragments of the just rediscovered sarcoplasmic reticulum. After we had observed that the relaxing factor was only transiently inactivated by small quantities of calcium ions, we intended to find out what had occurred to the added calcium. Using radioactive calcium, we found that the calcium had been completely taken up by the vesicles. We then demonstrated that the accumulation of calcium by the vesicles was a process causally linked to ATP hydrolysis. It was shown that during calcium accumulation an extra ATPase becomes active. Later we verified that the translocation of two calcium ions requires the splitting of one molecule of ATP. This analysis became possible only due to our trick of using oxalate at correct concentrations for trapping calcium ions inside the vesicles. It allowed us to estimate the calcium concentration gradient established by the transport system and therefore to prove that the calcium ions were transported uphill and, furthermore, to measure simultaneously calcium uptake and ATP splitting.

"The accumulation of calcium oxalate excludes that calcium removal was brought about by ATP-dependent calcium binding. On the other hand, due to the use of oxalate we were attacked by physiologists for producing physiologically irrelevant artifacts with unphysiological reagents. The biochemists were reserved and connected our findings with the simultaneously observed energy-dependent mitochondrial calcium uptake which in the long run proved to be of minor physiological importance. The priority of our finding was temporarily shadowed by the fact that one year later results of Ebashi and Lipmann[3] were published with the claim of also having demonstrated an ATP-dependent concentration of calcium, although their results only supported the retention of minute amounts of calcium by the membranes.

"The paper is highly cited because in the presence of ATP the sarcoplasmic calcium transport system had become, in conjunction with the calcium sensitivity of the contractile system pioneered by A. Weber[4] and Ebashi,[5] a basic element in our concept of excitation contraction coupling and, furthermore, a system most suitable for studying the chemical and structural events connected with ion translocation.[6] I received the Feldberg Award in 1963 for this work."

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3. **Ebashi S & Lipmann F.** Adenosine triphosphate-linked concentration of calcium ions in a particulate fraction of rabbit muscle. *J. Cell Biol.* 14:389-400, 1962.
4. **Weber A.** On the role of calcium in the activity of adenosine 5'-triphosphate hydrolysis by actomyosin. *J. Biol. Chem.* 234:2764-9, 1959.
5. **Ebashi S.** Third component participating in the superprecipitation of 'natural actomyosin.' *Nature* 200:1010, 1963.
6. **Hasselbach W.** Calcium-activated ATPase of the sarcoplasmic reticulum membranes. (Bonting S L & de Pont J J H H M, eds.) *Membrane transport.* Amsterdam: Elsevier/North-Holland, 1981. p. 183-208.