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This Week's Citation Classic®

Ebashi S. Calcium binding activity of vesicular relaxing factor.
J. Biochem. Tokyo 50:236-44, 1961.
[Rockefeller Institute, New York, NY]

This paper offered a quantitative basis for the Ca concept for muscle contraction, a concept that at that time had not been accepted by the majority. The relationship between Ca^{2+} concentration and the contraction of the actomyosin system had been extensively studied. Natural actomyosin, which had been deprived of most of its contractility as a result of careful washing to eliminate contaminating Ca, could be fully activated by a few μM Ca^{2+}, significant activation being seen even with 0.2 μM Ca^{2+}. This was the first indication of a physiologically effective Ca^{2+} concentration, which was later revealed to be common to intracellular processes of all the cells. It was also shown that the relaxing effects of chelating agents could be explained quantitatively by their removal of Ca^{2+} from actomyosin and that the natural relaxing factor, i.e., fragmented sarcoplasmic reticulum, could depress contraction according to its Ca^{2+} binding capacity. [The *SCI*® indicates that this paper has been cited in more than 525 publications.]

The First Correct Prediction

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The thought underlying the review article by Endo and me,[1] nominated as a *Citation Classic* in 1981, largely originated from the experimental results of this paper.

My accomplishments in Fritz Lipmann's laboratory at the Rockefeller Institute in 1959 might be divided into two categories: One was the discovery of ATP-dependent Ca uptake of the relaxing factor, which was shown to be derived from the sarcoplasmic reticulum.[2,3] The other was the finding that the contraction and relaxation of the actomyosin system was dependent on the rise and fall in Ca^{2+} concentration in the medium; the function of the relaxing factor, i.e., fragmented sarcoplasmic reticulum, could thus be explained solely by its Ca uptake.

In 1958 I had a chance to examine the relaxing effects of a series of chelating agents on glycerinated muscle fibers. Although I noticed the remarkable relaxing effect of EDTA,[4] a clear conclusion was only possible after my arriving at the Rockefeller Institute, where I was able to take advantage of its famous library. The result was the demonstration of a beautiful relationship between the Ca binding capacities of chelating agents and their relaxing activities.[2] This led me to postulate that the physiological relaxing factor must also bind Ca very strongly and the relaxation must be brought about by the removal of essential Ca from the actomyosin system.

The experimental results were just what I had expected,[1] which, in itself, was unusual because my earlier predictions had never been correct. I still well remember the day early in June 1959 when I conducted the first experiment and found that the relaxing factor beautifully bound Ca in the presence of ATP. I triumphantly told Professor Lipmann, expecting his praise, but I soon realized that I had been too optimistic. His negative response was partially due to his disbelief in Ca, a common view held by contemporary biochemists, but more important might have been my scientific attitude, i.e., my lack of inquiry into the core of the problem. From his critical eye, I was lacking in the mind of a biochemist.

The latter half of 1959 was dedicated to the acquisition of results that could persuade him to recognize Ca. Since the use of chelating agents was not generally accepted at that time, the necessity of Ca^{2+} had to be demonstrated by adding it in a net amount. For this everything had to be carefully washed to remove Ca^{2+} from the entire system, not only from the actomyosin system, but also from all chemical reagents involved. This was painstaking work, which I would not wish to have to do again.

Professor Lipmann was personally very kind and generous to me, accepting almost everything I proposed. As a result I was able to submit the manuscript to the *Journal of Biochemistry*, to which most of my papers had been contributed. Thus, the work done abroad was published in a Japanese journal. This thereafter became a principle that I have followed throughout my scientific career. Whether or not it is a good principle and whether or not I have profited from it, I have never tried to judge, but as far as the above article is concerned, it was the right decision. The paper might not have been accepted by a popular journal in the US or Europe; and, even if accepted, many portions, which, from my point of view, were critical, would probably have been altered or deleted by authoritative reviewers.

When asked to select one original paper from among my publications, it is always this article that I name without hesitation. This often appears rather puzzling to the questioner, who has expected that my choice would be a paper related to troponin. However, the discovery of troponin was rather a natural consequence of the Ca concept and could have been done by any scientist, perhaps even more elegantly. This article, on the other hand, was my fruit of nearly 10 years of inquiry into the mechanism of the relaxing factor and will remain indelibly imprinted in my memory.

1. **Ebashi S & Endo M.** Calcium ion and muscle contraction. *Progr. Biophys. Mol. Biol.* 18:123-83, 1968. (Cited 1,205 times.) [See also: **Ebashi S.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology.* Philadelphia: ISI Press, 1986. p. 257.]
2. **Ebashi S.** Calcium binding and relaxation in the actomyosin system. *J. Biochem. Tokyo* 48:150-1, 1960. (Cited 125 times.)
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. (Cited 575 times.)
4. **Ebashi S, Ebashi F & Fujie Y.** The effect of EDTA and its analogues on glycerinated muscle fibers and myosin adenosinetriphosphatase. *J. Biochem. Tokyo* 47:54-9, 1960. (Cited 30 times.)

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