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This Week's Citation Classic®

Harigaya S & Schwartz A. Rate of calcium binding and uptake in normal animal and failing human cardiac muscle: membrane vesicles (relaxing system) and mitochondria. *Circ. Res.* 25:781-94, 1969.
[Division of Myocardial Biology, Baylor College of Medicine, Houston, TX]

This paper describes a rapid method for the isolation of membranes derived from the sarcoplasmic reticulum, so-called "relaxing factors," from the cardiac muscle of human, rabbit, and dog and also from the white and red muscle of the rabbit. Comparative studies on Ca transport in these membrane vesicles are conducted by a dual-beam spectrophotometric assay procedure. [The *SCI*® indicates that this paper has been cited in over 455 publications.]

Shoichi Harigaya
Biological Research Laboratory
Tanabe Seiyaku Co., Ltd.
Toda, Saitama 335
Japan
and
Arnold Schwartz
Department of Pharmacology and
Cell Biophysics
College of Medicine
University of Cincinnati
Cincinnati, OH 45267-0575

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In 1967 most investigators who wanted to prepare "cardiac-relaxing factors" (sarcoplasmic reticulum membranes) experienced great difficulty in getting a highly active, stable preparation. Although it was generally accepted that the basic mechanism for relaxation of cardiac muscle[1-3] was qualitatively the same as that of skeletal muscle, precise studies of Ca binding and uptake by cardiac-relaxing factor were not carried out because of the difficulty in preparing membranes. We decided to try to establish a recipe for preparing membranes that could be easily duplicated in any laboratory.

Just before Harigaya came to Schwartz's laboratory in 1967, he had succeeded in isolating a high-activity "relaxing factor" from red muscle[4] in Ebashi's laboratory.

A series of lucky events, therefore, encouraged us to persevere and finally succeed in concocting a recipe that is quick and easy, which probably accounts for the number of citations.

Over a period of six months we struggled with several types of recipes and homogenization techniques. We repeated the preparation with small modifications every day. Finally, we decided to try a new homogenizer, the Polytron, which Schwartz and Sordahl had used in isolating a preparation of mitochondria.[5] The use of the Polytron proved to be an essential first step for the success of our preparation method. The Polytron caused a type of shearing of the tissue and, together with a slight sonication, was able to gently remove the membranes from the contractile proteins.

We used rabbit heart for the membrane source, although we had known from our experiments that rabbit heart was one of the most difficult tissues from which to get a good preparation compared with the same tissue from other animals. It was, however, good for us because we could more easily get a highly active preparation (at this time, at least) from the hearts of other animals or from other types of muscles. We could carry out precise, quantitative, comparative studies using this method with the white and red skeletal muscle and cardiac muscle, as well as with the cardiac muscle of other various kinds of animals.

In addition, we enjoyed two other fortunate occurrences. One was that we acquired a dual-beam spectrophotometer. By the use of murexide, a calcium colorimetric reagent, we were able to compare the kinetics of Ca binding and uptake (really transport) of various muscle preparations.[6] The second event that stimulated our interest was that the heart transplant "season" began in Houston with DeBakey and Cooley at about the time we started our research. They routinely sent us fresh human heart muscle obtained at the time of operation from the transplant recipients. These preparations exhibited a slower rate of Ca accumulation and very little Ca release, similar to that of slow red muscle. Procedures for isolating and purifying sarcoplasmic reticulum Ca^{2+}-ATPase from cardiac and skeletal muscle are now well developed.[7]

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2. **Weber A, Herz R & Reiss I.** Nature of the cardiac relaxing factor. *Biochim. Biophys. Acta* 131:188-94, 1967. (Cited 55 times.)
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4. **Harigaya S, Ogawa Y & Sugita H.** Calcium binding activity of microsomal fraction of rabbit red muscle. *J. Biochemistry* 63:324-31, 1968.
5. **Sordahl L A & Schwartz A.** Effect of dipyridamole on heart muscle mitochondria. *Mol. Pharmacol.* 3:509-15, 1967. (Cited 65 times.)
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7. **Nakamura J, Wang T, Tsai L-I & Schwartz A.** Properties and characterization of a highly purified sarcoplasmic reticulum Ca^{2+}-ATPase from dog cardiac and rabbit skeletal muscle. *J. Biol. Chem.* 52:5079-83, 1983.

