This Week's Citation Classic®

Reuter H & Seitz N. The dependence of calcium efflux from cardiac muscle on temperature and external ion composition. *J. Physiol.—London* 195:451-70, 1968.
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Calcium extrusion from heart muscle depends on the electrochemical sodium gradient across the membrane. One Ca and two Na ions compete for a "carrier" site at the external surface of the membrane. This was the first paper that provided direct evidence for Na/Ca countertransport (Na/Ca exchange) in the heart. [The *SCI*® indicates that this paper has been cited in over 675 publications.]

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I became interested in the question of Ca extrusion from heart cells after finding that Ca influx contributes a current to the plateau phase of the cardiac action potential.[1] In 1966 I participated in a laboratory course on "membrane biophysics" organized by H. Passow and R. Stämpfli in Homburg/Saar. The students had to do tracer flux experiments in frog muscle, which showed an electroneutral Na/Na exchange that was explained by a Na-loaded "carrier" in the membrane, energetically driven by the electrochemical Na gradient. H.H. Ussing had called this phenomenon "exchange diffusion."[2] Here I got the idea that not only might Na exchange for Na, but also for Ca. In contrast to Na/Na exchange, Na/Ca exchange could provide net transport of both ions—for example, by exchanging extracellular Na for intracellular Ca.

As soon as I was back in the Department of Pharmacology, University of Mainz, where I worked at that time, I started to do ^{45}Ca efflux experiments in guinea pig auricles to test this hypothesis. A colleague, Norbert Seitz, helped me with the rather tedious experiments. He left science shortly afterwards and went into general medical practice. Soon we discovered that ^{45}Ca efflux was greatly reduced when Ca and Na were eliminated from the bathing medium. Metabolic inhibitors increased rather than decreased Ca efflux and, in agreement with an exchange diffusion process, its temperature dependence was rather low. We divided the total ^{45}Ca efflux into a Ca- and a Na-activated fraction and, depending upon the ratio $[Ca^{2+}]/[Na^{+}]^2$ in the bathing medium,[3] we showed competition between both components. A similar competition was later found in cardiac membrane vesicles.[4] However, we were misled by this relationship in terms of the true stoichiometry of the exchanger. We proposed a 2Na:1Ca exchange. This is energetically not enough to reduce the free intracellular Ca concentration (unknown at that time) to 0.1 μM or less. Today a 3:1 exchange stoichiometry is fairly well established.[4] Slightly later, together with H.G. Glitsch and H. Scholz, we found that ^{45}Ca influx depended on the intracellular Na concentration, indicating that the exchange system worked in both directions.[5]

At the same time, P.F. Baker, M.P. Blaustein, A.L. Hodgkin, and R.A. Steinhardt were working in Plymouth on the same problem in squid axons.[6] Neither group was aware of this "competition," and, later on, very friendly relationships developed between us. Blaustein has described this part of the Na/Ca-exchange story in another *Citation Classic.*[7]

In October 1967 we submitted our paper, the second I had written in English, to the *Journal of Physiology*, and, to our joy, it was quickly accepted without revision. In the following years much of the quantitative work was done on internally perfused or dialyzed squid axons, while progress in cardiac muscle was slow. This changed when J.P. Reeves and colleagues developed a vesicular membrane preparation from cardiac muscle. Extensive work on the kinetics, energetics, and stoichiometry of Na/Ca exchange has since been done on this preparation.[4]

The article continues to be highly cited probably because it was the first full-length paper on this subject. The interest in the control of free intracellular Ca as a second messenger has increased enormously over the years. Na/Ca exchange is one of the Ca-transport systems that, in many cells, is heavily involved in this control.

1. **Reuter H.** The dependence of slow inward current in Purkinje fibres on the extracellular calcium-concentration. *J. Physiol.—London* 192:479-92, 1967. (Cited 355 times.)
2. **Ussing H H & Zerahan K.** Active transport of sodium as the source of electric current in the short-circuited isolated frog skin. *Acta Physiol. Scand.* 23:110-27, 1951. (Cited 1,730 times since 1955.) [See also: **Ussing H.** Citation Classic. (Barrett J T, ed.) *Contemporary classics in the life sciences. Volume 1: cell biology.* Philadelphia: ISI Press, 1986. p. 281.]
3. **Wilbrandt W & Koller H.** Die Calciumwirkung am Froschherzen als Funktion des Ionengleichgewichts zwischen Zellmembran und Umgebung (Effects of calcium on frog heart as a function of ion equilibrium between cell membrane and environment). *Helv. Physiol. Pharmacol. Acta* 6:208-21, 1948. (Cited 160 times since 1955.)
4. **Reeves J P.** The sarcolemmal sodium-calcium exchange. *Curr. Topics Mem. Transp.* 25:77-127, 1985. (Cited 5 times.)
5. **Glitsch H G, Reuter H & Scholz H.** The effect of the internal sodium concentration on calcium fluxes in isolated guinea-pig auricles. *J. Physiol.—London* 209:25-43, 1970. (Cited 290 times.)
6. **Baker P F, Blaustein M P, Hodgkin A L & Steinhardt R A.** The influence of calcium on sodium efflux in squid axons. *J. Physiol.—London* 200:431-58, 1969. (Cited 650 times.)
7. **Blaustein M P.** The interrelationship between sodium and calcium fluxes across cell membranes. *Rev. Physiol. Biochem. Pharmacol.* 70:33-82, 1974. (Cited 535 times.) [See also: **Blaustein M P.** Citation Classic. *Current Contents/Life Sciences* 30(35):14, 31 August 1987.]

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