between viviparity in recent sharks and distribution geographically (tropical versus temperate) or by depth of habitat (benthic versus pelagic). Finally, Wourms justifiably dismissed purported osmoregulatory considerations. Little is known about the food habits or feeding ecology of sharks during ontogeny except that the young are frequently found in different places than the more migratory adults. The suggestion that oophagy produces a predator trained before birth is not acceptable for holocephalans, among which no known members can be considered predacious. However, there is a strong correlation of viviparity with large adult size, which in turn has implications for feeding ecology. Viviparous species produce not only larger offspring but also fewer of them. Large size is an absolute advantage in reducing the number of potential predators. Fewness in the number of young is an absolute advantage in reducing competition for food among them. Large size and low numbers may have been a particularly advantageous combination in a community such as Bear Gulch, where the extreme diversity of the chondrichthyans indicates very fine-grained partitioning of resources based in part on size and age segregation (*5*).

Chondrichthyan viviparity has evolved independently many times, and must be seen as of considerable selective advantage by virtue of, rather than in spite of, the production of fewer, larger young. The probable evolution of viviparity with dental specializations for intrauterine feeding in a Paleozoic holocephalan reinforces the suggestion that this is evolutionarily the simplest method of viviparity, requiring no specialized maternal structures and few fetal modifications.

RICHARD LUND

Department of Biology,
Adelphi University,
Garden City, New York 11530

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Sodium-Calcium Exchange in Rabbit Heart Muscle Cells: Direct Measurement of Sarcoplasmic Ca^{2+} Activity

Abstract. *Calcium ion-selective microelectrodes made with Simon's neutral carrier were used to measure simultaneously sarcoplasmic Ca^{2+} activity (a^i_{Ca}) and resting tension (T_r) of rabbit ventricular muscle during reduction and restoration of external sodium ion concentration, $[Na]_o$. Under the same experimental conditions the change in contractile tension (T_a) also was measured. In resting muscle the a^i_{Ca} was 38 ± 17 nanomolar (mean ± standard deviation; N = 10). The reduction of $[Na]_o$ from 153 to 20 millimolar led to about a threefold increase in a^i_{Ca} with parallel increases in T_r and T_a. The time course of the change in a^i_{Ca} was similar to that of the changes in T_r and T_a. The results are consistent with an important role of the sodium-calcium exchange system for regulating sarcoplasmic Ca^{2+} activity.*

The concentration of calcium ions in the sarcoplasm is critical in the regulation of muscle contraction. Although sarcoplasmic Ca^{2+} concentrations of resting heart muscle appear low, they have not been measured directly. In this study, we measured the sarcoplasmic Ca^{2+} activity of rabbit heart muscle with Ca^{2+}-selective microelectrodes. To test the occurrence of a sodium-calcium exchange across the sarcolemma, we measured simultaneously the changes in sarcoplasmic Ca^{2+} activity and resting tension of the heart muscle during reduction and restoration of external Na^+ concentration. This maneuver is thought to diminish Na^+ influx and hence Ca^{2+} efflux. Thus, sarcoplasmic Ca^{2+} activity is expected to increase.

The Ca^{2+}-selective microelectrodes were made with a Ca^{2+}-selective liquid provided by Simon (*1*). Glass micropipettes with tip diameters of about 1 μm or less were made from borosilicate glass capillaries (Corning, code 774). The surface of the micropipettes was silanized by exposure to dichlorodimethylsilane gas. The micropipettes were filled with 100 m*M* $CaCl_2$ solution, and then by suction they were filled with the Ca^{2+}-selective liquid up to 400 to 800 μm from the tip. The right panel of Fig. 1 shows the potential recordings measured at the Ca^{2+} concentrations of 10^{-8} to 10^{-3}*M*; the left panel shows the measured potentials plotted against Ca^{2+} activities. We presented the results as Ca^{2+} activities rather than Ca^{2+} concentrations because

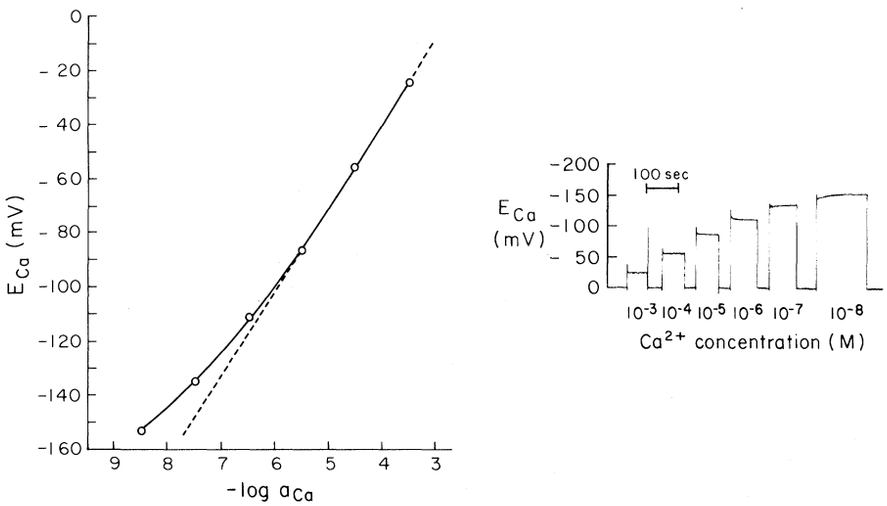

Fig. 1. Calibration of a Ca^{2+}-selective microelectrode. For the microelectrode calibration, solutions of 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}, 10^{-4}, and 10^{-3}*M* Ca^{2+} concentration were used. Those containing 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5}*M* Ca^{2+} were made from a Ca^{2+}-buffer containing EGTA. The Ca^{2+} concentrations of the solutions were calculated with the apparent stability constant of $5.76 \times 10^6 M^{-1}$ for Ca-EGTA at *p*H 7.0. The solutions containing 10^{-4} and 10^{-3}*M* Ca^{2+} were made by dilution of 0.1*M* $CaCl_2$ solution. All the solutions contained 150 m*M* K^+ and 1 m*M* Mg^{2+}, and had a *p*H of 7.0. The solutions have similar ionic strength; therefore, the calibration solutions have similar activity coefficients of Ca^{2+}. Bates and Alfenaar (*7*) have described the conventions for determining single ion activity coefficients for various ions including those of calcium. In the mixture containing $CaCl_2$ and KCl or NaCl, the activity coefficients of Ca^{2+} at various ionic strengths have been reported (*8*) and can be calculated by the extended Debye-Huckel equation (*9*). The experimental values agree with the calculated values. At the ionic strength of 0.15, the activity coefficient of Ca^{2+} (calculated) was 0.32. The microelectrodes were calibrated before and after each experiment. The properties of Ca^{2+}-selective ligands including selectivities have been reported (*10*).

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(i) many electrophysiological processes of cells respond to the ion activities in the strict sense, (ii) the microelectrodes measure the ion activities, and (iii) to express the results as Ca^{2+} concentration, it must be assumed that the activity coefficient of Ca^{2+} in sarcoplasm is the same as that of extracellular solution. To determine Ca^{2+} activities in the calibration solutions, we used an activity coefficient of 0.32 for Ca^{2+}. At Ca^{2+} concentrations greater than $10^{-6}M$, the changes of the microelectrode potentials were linear, with Nernst slopes of about 30 mV for a tenfold change in Ca^{2+} concentration. Between 10^{-6} and $10^{-7}M$ Ca^{2+} concentrations the Nernst slope varied from 10 to 25 mV. Between 10^{-7} and $10^{-8}M$ Ca^{2+} concentrations the slope varied from 5 to 15 mV.

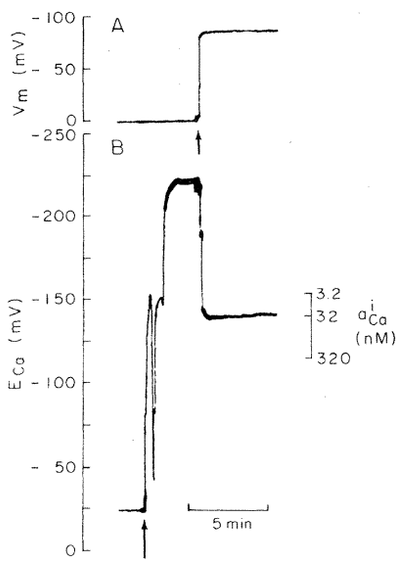

Fig. 2. Potential recordings of a conventional microelectrode (A) and a Ca^{2+}-selective microelectrode (B) for determination of sarcoplasmic Ca^{2+} activity. The impalement site of the conventional microelectrode was about 1 mm from the impalement site of the Ca^{2+}-selective microelectrode. Papillary muscles isolated from rabbit right ventricles were mounted in a narrow channel in a muscle chamber. The muscle chamber and the preparation of Tyrode's solution have been described (*5*, *6*). Tyrode's solution containing 1.8 m*M* Ca^{2+} was continuously perfused through the narrow channel. Tyrode's solution was saturated with a gas mixture of 95 percent O_2 and 5 percent CO_2 to achieve a *p*H of 7.2 to 7.4. All the experiments were carried out at 35° to 36°C. The tendon at the tapering end of the muscle was tied with one end of fine surgical silk; the other end of the silk was connected to a pin of a mechanoelectric transducer by means of a small ring. The other end of the muscle was held by a fine pin on paraffin in the narrow channel. The muscles were 0.7 to 1 mm in diameter and 5 to 7 mm in length. The techniques and criteria used for measuring transmembrane potentials with conventional and ion-selective microelectrodes have been described (*5*, *6*).

Figure 2 shows the sarcoplasmic Ca^{2+} activity of resting muscle perfused with normal Tyrode's solution. In Fig. 2A the cell membrane potential (V_m) was measured with a conventional microelectrode; in Fig. 2B the intracellular potential (E_{Ca}) was measured with a Ca^{2+}-selective microelectrode, and V_m was subtracted from E_{Ca}. The impalement of a cell with the Ca^{2+}-selective microelectrode resulted in a large potential change of about 200 mV. During impalements unstable potentials were often seen, probably reflecting temporary shunts. When the E_{Ca} reached a stable level, a cell was impaled with a conventional microelectrode (see arrow in Fig. 2A) at which time the E_{Ca} registered in Fig. 2B was rapidly changed by the same magnitude as the cell membrane potential and reached another stable potential. The rapid change in E_{Ca} represents the electronic subtraction of V_m from E_{Ca}. The E_{Ca} after the subtraction represents the sarcoplasmic Ca^{2+} activity. The equivalent scale of sarcoplasmic Ca^{2+} activities can be determined from the microelectrode calibration and is shown in Fig. 2B. With such measurements, the sarcoplasmic Ca^{2+} activity in the resting muscle averaged 38 ± 17 n*M* [mean ± standard deviation (S.D.) N = 10]. In these measurements, the resting membrane potential was −80.6 ± 3.5 mV (mean ± S.D., N = 10).

Figure 3A shows that after $[Na]_o$ was reduced from 153 to 20 m*M*, the resting membrane potential was depolarized by a few millivolts. After the reduction of $[Na]_o$, sarcoplasmic Ca^{2+} activity, a^i_{Ca}, increased and reached a relatively stable level. In four successful measurements, a^i_{Ca} increased from 45 ± 19 to 151 ± 52 n*M* (mean ± S.D.). The change in a^i_{Ca} (Fig. 3B) is equivalent to the potential change of about 10 mV. After the restoration of $[Na]_o$, V_m and a^i_{Ca} returned to almost their initial values. During the reduction and restoration of $[Na]_o$, the time course of the change in resting tension (Fig. 3C) is similar to that of the changes in a^i_{Ca} (Fig. 3B). This demonstrates a correlation between a^i_{Ca} and tension by a direct measurement of a^i_{Ca} in intact heart muscle cell.

Figure 3D shows that after the reduction of $[Na]_o$, the peak of contractile tension increased and reached a relatively stable level. After the restoration of $[Na]_o$, the peak of contractile tension decreased and returned to a level similar to that prior to the reduction of $[Na]_o$. The time course of the change in the contractile peak tension is approximately similar to that of the changes in a^i_{Ca} and resting tension. Although the time course of the change in a^i_{Ca} parallels that of the changes in tension, the two time courses may not be identical because of the time taken by Na^+ diffusion in extracellular space. The a^i_{Ca} was measured from a surface cell of the muscle, while the tensions were measured from the whole muscle.

Figure 3D also shows that during the low $[Na]_o$ period the diastolic tension slightly increased. This is consistent with the increase in the resting tension. The increase in contractile peak tension is much greater than that in resting tension. The increase in the peak tension (Fig. 3D) indicates that in the low $[Na]_o$ the amount of Ca^{2+} in sarcoplasmic reticulum that could be released increased rather than decreased. This is an indication that the increase in a^i_{Ca} is due to Ca^{2+} transport across sarcolemma from extracellular fluid. Thus, the increase in a^i_{Ca} and tension during low $[Na]_o$ provides evidence that the sodium-calcium exchange system located at the sarcolemma plays an important role in the contractility change by regulating sarcoplasmic Ca^{2+} activity and the Ca^{2+} content of the sarcoplasmic reticulum. However, no direct evidence is given that Ca^{2+} in sarcoplasmic reticulum increases during exposure to low $[Na]_o$. Therefore, we cannot exclude the possibility that

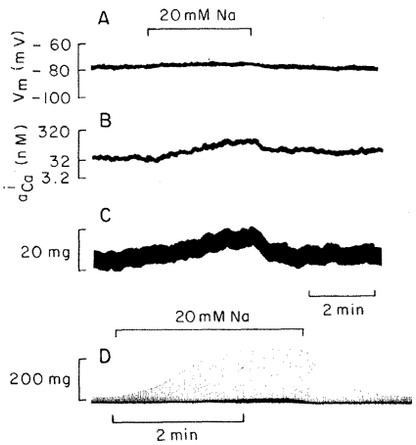

Fig. 3. Recordings of the simultaneous measurements of membrane potential (A), sarcoplasmic Ca^{2+} activity (B), and resting tension (C) during the alteration of extracellular Na^+ concentration $[Na]_o$. Under the same experimental conditions, contractile (twitch) tension (D) was separately measured from the same muscle. The experimental arrangement for tension measurement and stimulation has been described (*6*). The initial tension of the muscle was determined by stretching the muscle; the muscle length was then kept constant throughout the experiment. The tension transducer was linear with a sensitivity of 1.1 mV/mg. The solution containing low Na^+ concentration was made by substituting equimolar choline chloride for NaCl. Three additional experiments showed similar results. The stimulus rate was 60 per minute.

the increase in a^{i}_{Ca} of the resting muscle may be due to the release of Ca^{2+} from the sarcoplasmic reticulum or to some other mechanism not related to a sodium-calcium exchange at the sarcolemma.

This study shows that the a^{i}_{Ca} of ventricular muscle cells is 38 n*M* (mean value). This value is equivalent to a sarcoplasmic-free Ca^{2+} concentration of 119 n*M* if one assumes that the activity coefficient of Ca^{2+} in the sarcoplasm is the same as that of extracellular solution (0.32). This assumption is justified provided that the sarcoplasmic ionic strength is not too different from that of the extracellular solution. The value of 119 n*M* is similar to a sarcoplasmic-free Ca^{2+} concentration (*2*) of sheep cardiac Purkinje fibers. The value is also consistent with the range of the sarcoplasmic Ca^{2+} concentrations expected from actin-myosin interaction and the threshold for tension development (*3*). The measurement of a^{i}_{Ca} enables one to calculate that the Ca^{2+} equilibrium potential across the cell membrane is +128 mV. The results we obtained by changing $[Na]_o$ are consistent with those observed by other investigators (*4*). They show that Ca^{2+} movements across sarcolemma depend on both the external and the internal Na^+ concentration. Glitsch *et al.* (*4*) observed that their results are compatible with the idea of an exchange ratio of two sodium ions for each calcium ion and that the energy required for calcium extrusion by the Na-Ca exchange system seems to be provided by the Na gradient across the sarcolemma rather than by metabolic energy. If two sodium ions exchange for each calcium ion (electroneutrality), at equilibrium state the following relation is given: $(a^{i}_{Na})^2/(a^{o}_{Na})^2 = (a^{i}_{Ca})/(a^{o}_{Ca})$. If the ratios are calculated with the a^{i}_{Ca} measured in this study and the a^{i}_{Na} measured by Lee and Fozzard (*5*), $(a^{i}_{Na})^2/(a^{o}_{Na})^2$ is at least an order of magnitude greater than $(a^{i}_{Ca})/(a^{o}_{Ca})$. Whatever the exchange ratio of Na^+ and Ca^{2+} is, this study shows that the sarcoplasmic Ca^{2+} activity was increased by a reduction in the Na^+ activity gradient across the sarcolemma. This is consistent with the close correlation between contractility and intracellular Na^+ activity of sheep cardiac Purkinje fibers in which the Na^+ activity gradient was reduced by an increase in the intracellular Na^+ activity (*6*).

CHIN O. LEE
DAE Y. UHM
KARL DRESDNER
Department of Physiology,
Cornell University Medical College,
New York 10021

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Estrogen and the Growth of Breast Cancer: New Evidence Suggests Indirect Action

Abstract. *The growth of the MCF-7 human breast cancer cell line is unresponsive to the presence of estrogen in culture media. Paradoxically, in nude mice, growth of these cells and formation of solid tumors are dependent on estrogen. Tumors fail to develop in ovariectomized mice, but do develop in intact mice and in ovariectomized mice given estrogen. Primary cultures derived from MCF-7 tumors revert to unresponsiveness to estrogen. However, when these cultures are again transplanted into nude mice, estrogen is required for tumor formation. The continuous culture, the solid tumor, and the primary cultures therefrom have similar estrogen-binding capacities and affinities. These results indicate that mammary carcinoma cell growth in vivo is subject to inhibition that can be overcome by estrogen.*

Regression of human mammary carcinoma after ovariectomy has been linked to the presence of an estrogen receptor in the tumor (*1*). Indeed, tumors lacking the estrogen receptor seldom regress after ovariectomy. The presence of an estrogen receptor, however, does not always guarantee that ovariectomy will induce regression; about half of the tumors with estrogen receptors do not regress after ovariectomy (*2*).

Estrogen is believed to act on mammary cells by binding to the receptor (*3*). The influence of the estrogen-receptor complex on the growth of mammary epithelium is, however, not well understood. We therefore studied the effects of 17β-estradiol and insulin on the growth of the MCF-7 human mammary carcinoma cell line (*4*) in vitro and in vivo. Despite the presence of estrogen receptor at comparable levels in vitro and in vivo, MCF-7 cells grew without estrogen in vitro, but could not grow in vivo unless estrogen was present. In contrast, insulin, which also acts by binding to its receptor, enhanced the growth of MCF-7 cells both in vitro and in vivo. These findings have been interpreted to indicate that the effect of estrogen and its receptor on mammary cell growth in vivo must involve additional intermediary factors.

Soule and McGrath (*5*) reported that 4 weeks after MCF-7 cells were inoculated into 30 Swiss nude mice given supplementary estrogen, 70 percent developed tumors. The karyotypes of the tumor cell cultures derived from the nude mice were human and contained chromosomal markers characteristic of the MCF-7 cells in the continuous culture. In the present study, within 7 days after 2×10^6 actively growing (log phase) MCF-7 cells were inoculated into the mammary fat pad of each of 142 intact, athymic nude mice, BALB/c strain, 100 percent developed tumors. Tumors did not develop in ovariectomized mice or in mice made diabetic with streptozotocin (*6*). This failure could not be overcome even when the inoculum was increased to 20×10^6 cells for each mouse and when a latency period of 90 days was allowed. Tumors were obtained with 100 percent frequency and within 7 days in ovariectomized mice given a single injection of 0.1 mg of estradiol valerate at the time of cell inoculation. Similarly, tumors were obtained when each of the ovariectomized nude mice was given 10 μg of 17β-estradiol in the form of a pellet placed subcutaneously in the interscapular region. Tumors were also obtained with 100 percent frequency in nude mice made diabetic and given injections of insulin (0.2 I.U.) daily for 2 weeks.

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