tures failed to proliferate due possibly to a low seeding rate. 2 weeks after the cultures were set up, a closely woven network of epithelioid cells formed around open areas usually containing round granular macrophage-like cells. These macrophage-like cells had 1 or 2 bean-shaped nuclei, were acid phosphatase (fig.) and non-specific esterase positive and phagocytosed opsonized sheep red blood cells. The epithelioid cells were negative for the 2 enzymes and did not phagocytose. They had branching processes and pale staining nuclei with 2 nucleoli. Fat cell colonies were often formed at about 4 weeks.

Cell fractionation was carried out in an attempt to determine whether epithelioid and macrophage-like cells corresponded to the recipient and donor GPI contributions. Phagocytosis of carbonyl iron[6] was used to separate the phagocytic populations. The results are shown in table 3. Cells from stromal cultures that had taken up carbonyl iron were 91% donor GPI whereas cells remaining after treatment were 70% donor GPI. Some of this latter donor GPI was due to macrophage-like cells that were not extracted by this regime but despite the higher GPI specific activity of the iron fraction, residual macrophage-like cells would probably not account for all the donor GPI in the non-iron fraction. Friedenstein et al.[7] and Golde et al.[3] have concluded that bone marrow fibroblasts from mice and men are recipient in origin in radiochimeras. However, in vitro establishment of hematopoietic cultures using these cells as the microenvironment was not described. Keating et al.[2] claimed that the in vitro microenvironment was donor in origin in a study of cultured bone marrow from human patients treated with cyclophosphamide, radiation and bone marrow transplant.

Thus, while it appears that there is a mesenchymally derived cell that may not be replaced by bone marrow transplant, the question remains is this the cell that permits hematopoiesis in vitro. Our results indicate that a substantial part of stroma of mouse bone marrow cultures is donor derived. That it is also derived from a hematopoietic stem cell is suggested by our failure to detect any evidence of hematopoiesis or transfer of donor cells from primary stromal cultures (table 1). In vivo in the chimera colonization of the mechanocytic framework of the host by new donor-derived microenvironment-macrophagic and epithelioid cells may take some time to manifest itself.

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External strontium and contractility in single giant muscle fibers of the barnacle

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Summary. Isometric tension and membrane potential in response to electrical stimulation have been studied on single giant muscle fibers of the barnacle upon replacement of external calcium with strontium. Under these conditions, the membrane response showed after the normal peak depolarization, a plateau phase lasting 3-7 sec before repolarization took place, while the force of contraction showed a linear relation with external strontium concentration. A direct action of strontium ions on contractile proteins (namely troponin) can be ruled out in favor of a triggering action on the sarcoplasmic reticulum, which, in turn, leads to calcium release and development of tension.

In crustacean muscle fibers the activation of membrane Ca^{2+} channels, and the resulting inward Ca^{2+} current, normally initiates the electrical response[3,4] which represents the first step in the series of events leading to contraction[5]. However, in these fibers Ca^{2+} channels do not seem to be very selective for Ca^{2+}. Hagiwara and Naka[6] obtained action potentials in EDTA-treated fibers even when the external Ca^{2+} was completely replaced by Sr^{2+}, suggesting that this ion can pass through the membrane, probably through the channels carrying the inward current. Further studies using the voltage clamp technique have shown that the amplitude of the inward currents for Ca^{2+} and Sr^{2+}, respectively in Ca- and Sr-media, became maximal at the same membrane potential level, and their ratio was of the order of unity[7]. These reports have suggested the present work, which I have carried out to study the effect of replacement of external calcium with strontium on isometric tension and membrane potential responses in electrically stimulated single muscle fibers.

Methods. The experiments were done on single giant muscle fiber from the barnacle *Balanus nubilus.* These were isolated and cut at one end, and then firmly attached to a glass cannula through which the stimulating-recording electrode[8] was inserted. The fibers were set up vertically with conventional arrangements for recording tension with the RCA 5734 transducer. Tension output was in the range 1-10 Newton · cm^{-2} (2-20% T_{max}) to avoid the fibers being pulled away from the cannula. Isometric tension and membrane potential signals were displayed on a Tektronix storage oscilloscope, where the records could be readily photographed. The EGTA solutions were injected axially inside the fiber with a microsyringe having a glass capillary (100-120 μm diameter) attached to the stainless-steel needle. Details of the dissection, cannulation and experimental apparatus may be found in Ashley and Ridgway[8]. The artificial sea water (ASW) used contained (mM): NaCl 510.4, KCl 12.9, $MgCl_2$ 23.6, $CaCl_2$ 11.8, $NaHCO_3$ 2.6 and 2 mM-TES; pH was 7.25.

Results and discussion. In an attempt to understand to what extent Sr^{2+} can substitute for Ca^{2+} in the mechanism of contraction, isometric tension in response to test stimuli was recorded on fibers bathed in ASW, and after replacement of the solution either with 0Ca-ASW or 12 Sr-0Ca-ASW Neither in 0Ca-ASW nor in 12 Sr-0Ca-ASW was the resting membrane potential significantly changed, the average depolarization observed being 2-3 mV[9]. The

results shown in figure 1 indicate that the tension declined as a function of time in both experimental procedures, but their half times were markedly different. In addition, during 12Sr-0Ca-ASW perfusion, when the isometric tension approached values close to 5–3% of control (which occurred about 30 min after solution replacement), the electrical membrane response assumed a peculiar time course, as illustrated in figure 2. This consisted of the normal peak depolarization ('spike') followed by a plateau phase which kept the membrane potential at a steady level, close to the peak depolarization value, for 3–7 sec. A sudden repolarization brought the membrane potential to its resting level. With the appearance of this post-'spike' plateau phase (PSPP), isometric tension, which by this time had become negligible, returned to attain values comparable to controls; however, the time course was greatly different. Tension developed very slowly (maximal slope (dP/dt) was normally 0.2–1 Newton $\cdot$ cm^{-2} $\cdot$ sec^{-1}, compared with 8–20 Newton $\cdot$ cm^{-2} $\cdot$ sec^{-1} observed on ASW bathed fibers), with hundreds of msec delay from peak depolarization, and generally continued to increase after the membrane potential had returned to the resting value. Relaxation was equally slow. Even though we observed the PSPP response only in 12 SR-0Ca-ASW, a few fiber were tested to assess whether any effect due to calcium removal existed after longer treatment. Fibers were kept in 0Ca-ASW for 30 min (long after any mechanical response had disappeared), nevertheless no PSPP could be observed. Spikes were sometimes followed by oscillatory potentials, similar to those obtained on the same preparation by Hagiwara et al.[10] at a very low external calcium concentration, $[Ca^{2+}]_0$. They did not, however, induce any tension response. When Sr^{2+} was added to the medium, the PSPP together with the development of tension soon appeared. Electrical and mechanical responses were substantially similar to those obtained following the previous procedure (fig. 2) of directly replacing ASW with 12 SR-0Ca-ASW, as can be seen in figure 3a.

From these recordings, obtained at 2 different $[Sr^{2+}]_0$ a relation appears to exist between $[Sr^{2+}]_0$ and tension. To confirm this point, various $[Sr^{2+}]_0$ were tested; the results are shown in figure 3b. These results allow the assumption of an involvement of Sr^{2+} both in the contractile process a) Sr^{2+} slows down the rate of decline of tension in 0Ca-ASW medium; b) tension development during the PSPP seems to depend on $[Sr^{2+}]_0$), and in the onset of the PSPP (which was not observed in the absence of Sr^{2+} in the medium). (The apparent conflict between these results and those of Hidalgo et al.[11] who found no tension in barnacle fibers bathed in 0Ca-10SR-ASW media is very probably due to the peculiar time course of membrane potential (PSPP) observed in my experiments and described above.) From the evidence that Sr^{2+} ions enter the fiber in a very similar quantity to Ca^{2+} ions during the excitation[7], and can activate the contractile system[12] and bind to troponin (although with a lower affinity than Ca^{2+})[13], the tension response could simply be thought of as the activation of the contractile system by entrant Sr^{2+} ions, in this way justifying the relation between $[Sr^{2+}]_0$ and tension (fig. 3b). However, Sr^{2+} has also been shown to have a calcium-releasing action, both on pre-loaded SR vesicles[14], and on the SR of skinned fibers[15].

To gain further information on the role of Sr^{2+} on contractility, single muscle fibers were injected with 0.4 µl of either a 200 or 20 mM EGTA solution. The final concentration of the chelating agent inside the fiber (assuming uniform distribution, and 80 mg as average weight of the fibers) was 1 and 0.1 mM respectively. After 20 min equilibration in 0Ca-ASW, Sr^{2+} ions were added to the medium at different concentrations, ranging between 10 and 60 mM. While membrane response showed a PSPP no tension could be detected upon stimulation of the fiber 30 sec after Sr^{2+} addition. Even though the interpretation of the present results requires more information, as far as tension is concerned, a direct action of external Sr^{2+} on contractile proteins (namely troponin) seems to be ruled out. In fact, even when the final internal EGTA concentration in injected fibers in 1 mM, assuming the EGTA binding constant for Sr^{2+} to be 1/800[13] of that for Ca^{2+} ($[CaEGTA^{2-}]/[Ca^{2+}][EGTA^{4-}] = 1.0 \times 10^{11}$ M^{-1} (Schwarzenbach et al.[16]) the bulk of Sr^{2+} entering the fiber during activation would be expected to be available to interact on tension binding sites of troponin, hence generating tension. Therefore, if Sr^{2+} plays a major role in the sequence of events leading to

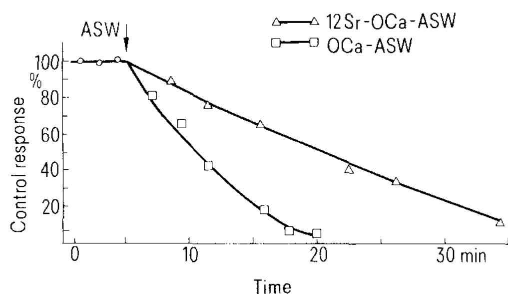

Figure 1. Reduction of contractile force in response to constant electrical stimulation, upon replacement (at the arrow) of ASW with either 0Ca-ASW (squares) or 12 Cr-0Ca-ASW (triangles). Tension is expressed as percentage of control value in ASW.

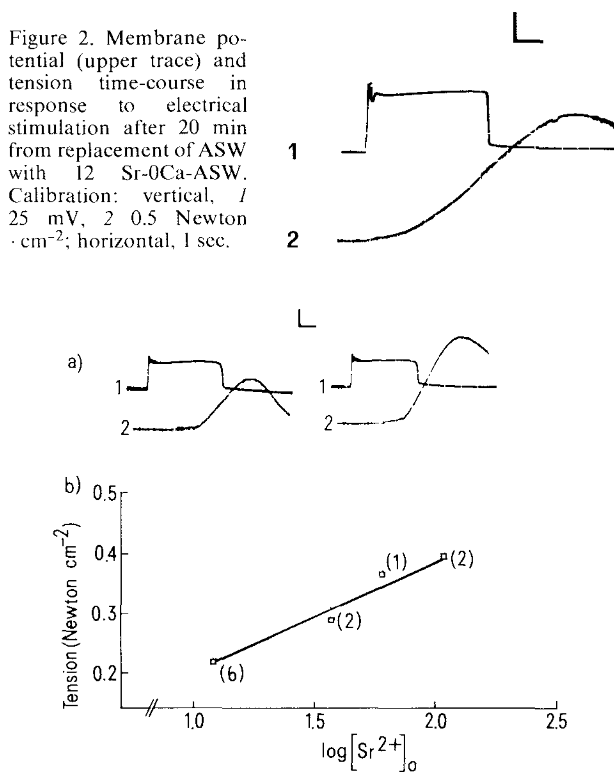

Figure 2. Membrane potential (upper trace) and tension time-course in response to electrical stimulation after 20 min from replacement of ASW with 12 Sr-0Ca-ASW. Calibration: vertical, *1* 25 mV, *2* 0.5 Newton $\cdot$ cm^{-2}; horizontal, 1 sec.

Figure 3. *a* Membrane potential (upper traces) and tension time course in 12 Sr-0Ca-ASW (left) and 60 Sr-0Ca-ASW (right) in response to constant electrical stimulation applied 30 sec after the addition of Sr^{2+}. The fibers were previously kept for 30 min in 0Ca-ASW. Calibration: vertical, *1* 30 mV, *2* 0.6 Newton $\cdot$ cm^{-2}; horizontal, 1 sec. *b* Relationship of mean isometric tension to $\log[Sr^{2+}]_0$ $\cdot$ $[Sr^{2+}]_0$ is in mM. The number of fibers is in parentheses.

contraction, beyond the likely action determining the PSPP, it is plausible to think of a triggering action on the sarcoplasmic reticulum which in turn leads to calcium release. (It has in fact been shown by Caputo and Di Polo[17], and Ashley et al.[18] that the calcium which activates the contractile system in barnacle fibers comes from intracellular stores, namely the sarcoplasmic reticulum.) Suarez-Kurtz and Sorenson[19] have come to a similar conclusion; they found a suppression of tension output when crab fibers bathed in either Ca^{2+} or Sr^{2+} media were treated with procaine, a suppressant of Ca^{2+} release from the sarcoplasmic reticulum.

From the evidence that EGTA-injected barnacle fibers can, in presence of Sr^{2+}, give rise to marked prolongation of the spike potential, it follows that: a) both Ca^{2+} removal and the presence of Sr^{2+} represent the conditions for the PSPP onset; b) the first condition seems to be attained either by 0Ca-ASW application or EGTA injection. With regard to the second point, Ca^{2+} removal is not to be interpreted as an intracellular calcium depletion, since 0Ca-ASW application has been shown to cause only a very little net loss of total intracellular calcium: less than 5% in the first hour[20]. However, no distinction was made among the various Ca^{2+} fractions inside the cell, and in which structures they are stored[21]. Therefore, it is difficult to recognize which calcium represents the net loss during 0Ca-ASW application, even though free myoplasmic Ca^{2+} is the most likely candidate.

As to the dependence of the development of the PSPP on the $[Ca^{2+}]_0$, a Ca-dependent membrane conductance effect may be tentatively considered. This view is supported by the observation that in leech neurons the increase in K-conductance after the spike requires the presence of external calcium[22]. Furthermore, the hypothesis that Ca^{2+} ions entering the fiber during the action potential are necessary for substained K-conductance finds also support from those experiments in which selective K-conductance increased upon intracellular injection of Ca^{2+}[23].

As far as the role played by Sr^{2+} in the PSPP onset is concerned, any interpretation would sound speculative because too little information is at present available. What has been shown is that Ba^{2+}, the other divalent ion which can enter the fiber during the activation and produce a spike in the absence of Ca^{2+}, can affect the membrane response in the way of producing PSPPs. While this contributes little to the understanding of the phenomenon, the evidence that the effect is not specific for a particular ion suggests that the adsorption of divalent ions on the outer side of the membrane could modify the field charge within it and so alter certain functions (namely, membrane conductance) mediated by the membrane.

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Excretion of acid hydrolases during molting in *Philosamia ricini*

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Summary. The level of the acid hydrolases β-glucuronidase (EC 3.2.3.11), acid phosphatase (EC 3.1.3.2) and acid protease (EC 3.4.4.) was studied during larval growth and molting in *P. ricini*. The level of activity of these enzymes remained low during larval growth; however, the level increased sharply at the time of molting and declined sharply thereafter in the newly ecdysed insect. Interestingly, the diminished activity of these enzymes was almost quantitatively recovered in the cast-off cuticle. The excretion of acid hydrolases through the cast-off cuticle has hitherto not been reported in insects during molting.

These observations suggest that during ecdysis the acid hydrolases are probably localized in the molting fluid present between the old and new cuticular layers. Since they cannot be transported to the hemolymph, they are excreted through the cast-off cuticle.

The level of acid hydrolases is known to increase during molting and metamorphosis in many insects[2–7]. The increase in acid hydrolase activity is an adaptive response to the autolytic or histolytic events occurring during metamorphosis. In insects, the fate of increased lysosomal enzymes once the histo-