pregnancy) indicated a further decrease in peripheral blood erythron indices and a more profound reduction in peripheral blood leukocytes and marrow granulocytic proliferative cells compared to normal values. The trauma of surgery to nonirradiated beagles in midtrimester of pregnancy resulted in a greater decrease of all peripheral blood indices, marrow nucleated erythroid cells, and marrow GM-CFCs. The decrease in all the peripheral blood parameters of irradiated pregnant beagles (14–22 days post-TBI) following surgery may have stimulated erythroid progenitor cell values to increase.

The compensatory role of the pregnant beagle bone marrow to support enhanced erythropoiesis following the trauma of TBI and surgery is suggested by the increase in CFU-Es and BFU-Es, most likely at the expense of granulopoiesis. Most of the traumatized pregnant dog GM-CFC values were below the normal dog values, whereas all traumatized pregnant CFU-E values were above the normal dog values. It is evident that a dose of 90 rad gamma radiation and the additional stress of surgery has a dramatic and sustained effect on pregnant canine bone marrow hemopoiesis. Evidence is accumulating regarding myelopoietic responses following surgical or wound trauma postirradiation[7-11]. Wiktor-Jedrzejczak[12] has recently reported enhanced hemopoietic stem cell activity in the postirradiated and bled animal. However, no apparent gross alterations were observed in uterine healing or in fetal growth in the contralateral uterine horn. In addition, no disruption in pregnancy was noted, however late the first surgery was performed.

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Change in the activation rate of voltage-dependent Ba^{2+} current by conditioning pre-depolarization

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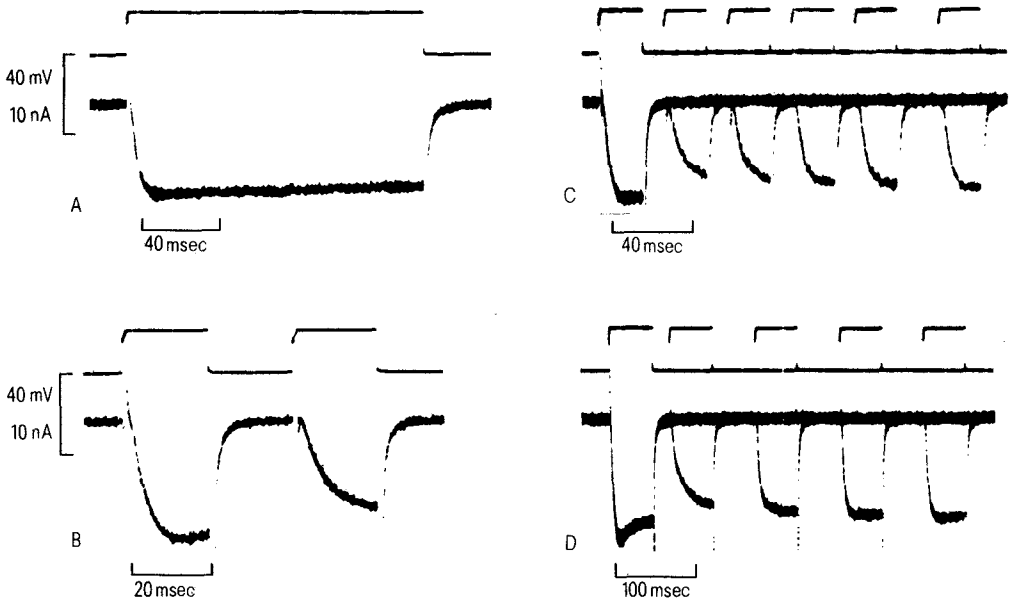
Summary. When Ba ions replace Ca ions in the external solution, conditioning depolarizing voltage-clamp pulses slow the activation rate of the fast early inward currents in the ciliate *Stylonychia,* while inactivation is greatly reduced in the presence of Ba.

The voltage-dependent Ca channel has been shown to be permeable to Ba and Sr ions[2]. In Ca-free Ba-solutions the early inward current is maintained at nearly its maximum amplitude during depolarizing pulses lasting for several hundred msec in many excitable cells, including ciliates[3-7]. This appears to be due to the blockade of the K outward current[7-9] and a reduction of inward current inactivation[7,10]. The use of Ba as charge carrier through the Ca channel therefore provides a favorable opportunity to study the activation kinetics of this channel. In molluscan neurones[3,11,12] and insect muscle[13] the activation of Ba (and Ca) currents is accelerated with increasing membrane depolarization. I have studied how the activation kinetics of the Ca channel in the presence of Ba ions as charge carriers are affected by a prepulse. The time constant of activation of Ba inward currents is increased by 60% by a given depolarizing prepulse.

The experiments were carried out on the hypotrich ciliate *Stylonychia mytilus.* The membrane was voltage-clamped with the technique using 2 intracellular microelectrodes described previously[6]. The membrane potential was held at -50 mV at its resting value. The membrane currents were not corrected for leakage current (< 1 nA) or capacitive currents, the time constant of the latter being smaller than 0.3 msec. The cells were first superfused in the standard Ca-solution containing 1 mM $CaCl_2$, 1 mM KCl and 1 mM Tris-Cl, pH 7.4. The test solution, containing 1 mM $BaCl_2$ instead of $CaCl_2$, was introduced while the cells were held in voltage-clamp to prevent repetitive excitation[6,7]. All experiments were performed at 17 ± 1 °C.

The figure shows 4 sample recordings of Ba inward currents following $+20$ mV (to -30 mV) voltage-clamp pulses. In the figure, A, a depolarizing pulse of 160 msec duration elicited an inward current, which achieved a maximum of 11 nA within 5 msec, and decayed to a near steady-state of 9 nA. Thus, there is relatively little inactivation of this net inward current in Ba as compared to inward currents in Ca[6]. When 2 voltage pulses were given (fig., B), the 2nd pulse evoked a current with a much slower rate of activation. The peak amplitude of the current following the 2nd-step pulse eventually achieved about 85% of the control current, but only within 20 to 25 msec (with an interval of



Ba inward currents (lower traces) following +20 mV (to −30 mV) voltage pulses (upper traces); *A* a single pulse of 160 msec duration; *B* 2 pulses of 20 msec duration separated by a 20-msec interval; *C* 5 sweeps of 2 pulses each, superimposed, the pulses lasting for 20 msec with an interval increasing from 10 to 150 msec; *D* 4 sweeps of 2 pulses each, superimposed, the pulses lasting for 50 msec with an interval increasing from 20 to 350 msec.

20 msec). The time-constant of activation, τ_m[14], increased from 1.63 to 2.28 msec in this experiment. The mean τ_m of the Ba inward current activation was 1.79±0.2 msec (±SD, n=7) for a step from −50 to −30 mV, and 1.30±0.07 msec (±SD, n=6) for a step to −20 mV. These figures agree well with the time constants of activation of Ba currents measured in internally perfused snail neurones[14]. In the figure, C and D are shown superimposed recordings of 2 pulses to −30 mV of either 20 msec (fig., C) or 50 msec (fig., D) duration, separated by an increasing interval. With the shortest interval given (10 msec) τ_m increased from 1.79±0.2 msec to 2.93±0.48 msec (±SD, n=5), which is a rise of 60%. There was no significant difference in these changes of τ_m with durations of the preceding pulse between 20 and 50 msec. With increasing intervals between the 2 pulses τ_m decreased with an exponential time course, its time constant ranging from 28 and 35 msec (n=4). In some experiments the test pulse to −30 mV and −20 mV was preceded by a hyperpolarizing conditioning pulse. Little or no change in the activation rate of the Ba inward current was observed with this pulse protocol.

The results show that pre-depolarization of the membrane slows the activation kinetics of the Ca channel, at least when Ba is the charge carrier. The effect of a depolarizing prepulse is 'memorized' for several tens of msec. The activation time constant of Ca inward currents also tends to increase when preceded by a depolarizing pulse[15], although this is more complicated to interpret due to the effects of Ca on Ca channel inactivation and K channel activation. Thus, the question remains unsettled, whether or not the activation rate of the Ca channel depends upon the permeating divalent cation. A small, opposite effect of pre-depolarizing pulses on the activation rate of Ba inward currents has recently been described for bovine chromaffin cells[16]. It appears from the present study that the intracellular accumulation of divalent cations may exert a negative effect on the rate of channel activation. As the voltage-dependent Ca channels in ciliates presumably reside in the ciliary membrane[17,18], accumulation of Ba (or Ca) in the small ciliary volume during maximum voltage-gated influx can be as much as 5×10^{-4} M or more[15]. This large increase in intraciliary Ba (Ca) concentration would be expected to change the surface potential of the membrane. Thus it is as yet uncertain, whether in the present experiments the change in activation rate of Ba inward currents is caused by a surface potential effect or by an altered gating process of the voltage-dependent channels. The latter mechanism has recently been suggested for the voltage-dependent Ca channel in *Paramecium*, when the inward current is carried by different divalent cations[19].

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