polarization slows the inherent rate of discharge, this cell increased its rate of spontaneous firing when it was hyperpolarized. Such behavior seems paradoxical but may be related to excessive inactivation of inward current at a low resting potential, which is then relieved by a hyperpolarizing pulse.

Spike electrogenesis was blocked by the addition of 4 m*M* $MnCl_2$ to the bath (Fig. 2, A–C), suggesting that these action potentials are Ca^{2+} spikes. Calcium spike electrogenesis appears to be a frequent correlate in cells that exhibit secretory behavior, cells as diverse as *Aplysia* bag cells (*5*) and rat pituitary cells (*1*). A gradual inactivation of the repolarizing current after a local depolarizing response (Fig. 2E) appeared when a depolarizing pulse of constant current and constant duration was repeated at a rate of 1 Hz. The depolarizing local potential elicited by the first pulse repolarized rapidly, and then the slope of the repolarization phase diminished stepwise with each of about six pulses, after which the repolarization phase became extinguished. The repolarization phase could be reactivated either by conditioning pulses of hyperpolarizing current or by a rest period. The process of inactivation of the repolarization phase was greatly accelerated in the presence of $MnCl_2$ (Fig. 2F). This suggests that a Ca^{2+}-activated K^+ gate may be involved in this process (*17*) or that the local response consists of a fast and a slow potential, the latter being dependent on readily available calcium. These two time-related phenomena—the switching of membrane potential and the inactivation of repolarization of the local response—may be examples of a dynamic electrophysiological state that exists in certain cells (*12*).

Although spike electrogenesis was long held to be peculiar to nerves and muscle, new investigations reveal the presence of electrogenesis in cells that are clearly not nerve or muscle. Our findings demonstrate the presence of a mechanism for spike generation, implicate calcium ions as the major charge carrier, and provide evidence for other forms of electrical activity in these lung tumor cells.

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13. The mean ± standard deviation (S.D.) of the input resistance (R_i) at the original membrane potential was 242 ± 155 megohms, whereas that of the switched membrane potential was 209 ± 167 (Table 1). In view of these data, we propose that switching is not the result of injury or conductance changes but may be due to a metabolic mechanism.
14. The steady-state current-voltage curves were linear in response to anodal pulses but exhibited delayed rectification in response to cathodal pulses. Test current pulses ranged from −3 nA to +3nA.
15. The postspike hyperpolarization was not voltage-dependent but rather was cell-specific, occurring in about one of every four cells examined.
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Divalent Cation Ionophores Stimulate Resorption and Inhibit DNA Synthesis in Cultured Fetal Rat Bone

Abstract. *Two divalent cation ionophores, A23187 and Ionomycin, which are selective for calcium, stimulated the resorption of fetal rat long bones in organ culture at 0.1 to 1 micromolar but not at higher concentrations. Both agents inhibited DNA synthesis at concentrations that stimulated resorption. These results might explain the differences in ionophore effects on bone previously reported, and they imply that cell replication is not required for osteoclast formation in fetal rat long bone cultures.*

Dziak and Stern (*1*) have shown that, like parathyroid hormone (PTH), the divalent cation ionophore A23187 stimulates resorption in cultured fetal rat long bones, enhances intracellular calcium entry, and increases adenosine 3′,5′-monophosphate (cyclic AMP) in cells isolated from fetal rat calvaria. Dietrich and Paddock (*2*) found that both A23187 and PTH inhibited the incorporation of tritiated proline into collagen in cultured newborn rat calvaria. However, in cultured newborn mouse calvaria, Ivey *et al.* (*3*) found that A23187 acts only as an inhibitor of bone resorption and has no effects on the accumulation of cyclic AMP in the medium. Differences between the action of PTH and A23187 have also been described in the rat. Hahn *et al.* (*4*) showed that calcitonin did not inhibit resorption stimulated by A23187 in fetal rat long bones, while Dietrich and Paddock (*2*) found that despite the similarities of their effects on collagen synthesis in fetal rat calvaria, A23187, unlike PTH, inhibited noncollagen protein synthesis and incorporation of tritiated thymidine into DNA. We have used 19-day fetal rat long bone cultures to compare the effects of PTH, A23187, and Ionomycin (*5*). Ionomycin, which is a divalent cation polyether ionophore previously untested on bone, is more selective for calcium than is A23187.

Bone resorption was assessed by measuring the release of previously incorporated ^{45}Ca (*6*). DNA and protein synthesis were assessed by measuring the incorporation of tritiated thymidine and tritiated amino acids, respectively. Long bone shafts labeled in utero with ^{45}Ca were cultured in 0.5 ml of modified BGJ medium (Gibco) supplemented with 5 percent fetal calf serum (Gibco) that was treated with dextran-coated charcoal to reduce endogenous bone resorbing activity (*7*). During the last 2 hours of culture, 1.5 μCi of tritiated thymidine or tritiated amino acid mixture (New England Nuclear) was added to each dish. At the end of the experiments bones were extracted in 5 percent trichloroacetic acid (TCA). The radioactivity from the ^{45}Ca was counted in the medium and TCA extract; the ^{3}H was counted in the TCA extracts and the bones after they were digested with NCS (Amersham). The ^{3}H counts were divided by the total ^{45}Ca counts of each bone to normalize for differences in fetal bone size. In most experiments, bones from the fetuses of a single litter were used to minimize variation caused by any nonuniformity of ^{45}Ca labeling. In some experiments the activity of the lysosomal enzyme β-glucuronidase was measured in the medium (*8*). For histologic studies the bones were placed in Millonig's fixative for 2 to 4 days and then dehydrated and embedded in meth-

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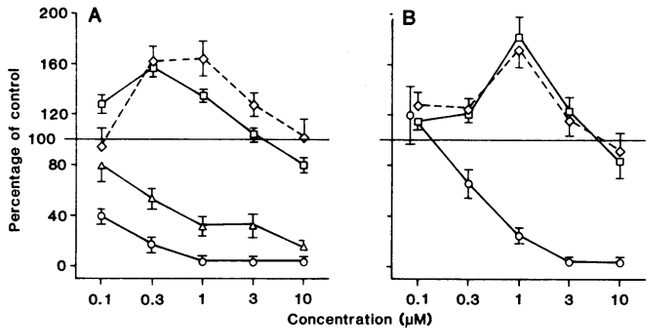


Fig. 1. Effects of various concentrations of (A) A23187 and (B) Ionomycin in cultures of fetal rat long bones on ^{45}Ca release (□), β-glucuronidase in the medium (◇), ^{3}H-labeled amino acid incorporation into bone (△), and [^{3}H]thymidine incorporation into bone (○). In (A), ^{45}Ca release is significantly increased over control ($P < .01$) at 0.1 μ*M* to 1.0 μ*M* but is significantly below control ($P < .01$) at 10.0 μ*M*; β-glucuronidase concentration is significantly increased over control ($P < .01$) at 0.03 μ*M* to 3 μ*M*; [^{3}H]thymidine incorporation is significantly inhibited compared to control ($P < .01$) at 0.1 μ*M* to 10 μ*M*, and [^{3}H]amino acid incorporation is significantly inhibited over control ($P < .01$) at 0.3 to 10 μ*M*. In (B), ^{45}Ca release is significantly increased over control only at 1 μ*M* ($P < .01$) as is β-glucuronidase concentration in the media ($P < .01$); [^{3}H]thymidine incorporation is significantly inhibited ($P < .01$) compared to control from 0.3 μ*M* to 10 μ*M*. Values are means ± standard error for 6 to 20 determinations.

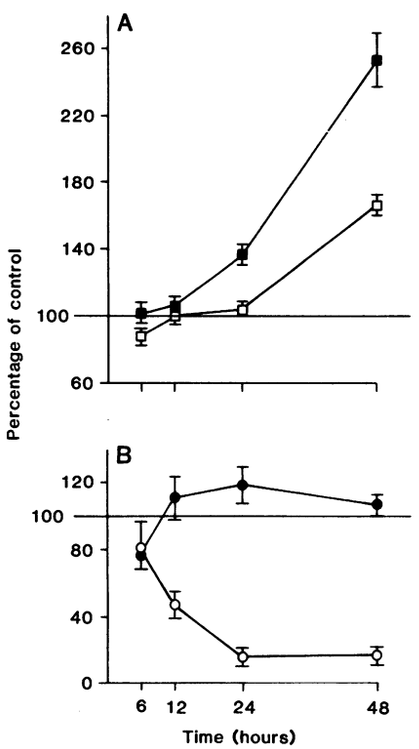
acrylate. Undecalcified sections (3 μm) were stained with a mixture of hematoxylin and crocein, and fuchsin. Statistical significance was determined by Student's *t*-test.

After 48 hours of treatment, A23187 increased ^{45}Ca release over a concentration range of 0.1 to 1.0 μ*M* with maximum resorption occurring at 0.3 μ*M* (Fig. 1A). This response was biphasic, and higher concentrations (10 μ*M*) inhibited ^{45}Ca release. A23187 decreased [^{3}H]thymidine incorporation in a dose-dependent manner. Incorporation of amino acids was also inhibited at concentrations of 0.3 μ*M* and greater, but this inhibition was less than that for thymidine. Ionomycin also increased ^{45}Ca release, but this effect was significant only at 1 μ*M* (Fig. 1B). Like A23187, Ionomycin caused a dose-dependent inhibition of thymidine incorporation. The effect of Ionomycin on amino acid incorporation was not determined. At maximally effective concentrations both ionophores increased bone resorption and inhibited thymidine incorporation to a similar degree. The decrease in thymidine incorporation produced by the ionophores appeared to be due to inhibition of DNA synthesis and not to alterations in uptake of the label, because the radioactivity from the [^{3}H]thymidine in the TCA extractable pool was not significantly altered by any concentration of ionophore tested. The changes are also not likely to be the result of variations in the specific activity of the precursor pool, since addition of unlabeled thymidine (0.1 m*M*) did not significantly alter the inhibitory effects.

The stimulation of ^{45}Ca release by the ionophores was accompanied by parallel increases in β-glucuronidase activity in the medium (Fig. 1). This effect was lost at the higher concentrations which caused less bone resorption.

The ionophore A23187 did not stimulate bone resorption as rapidly or to as great an extent as did PTH (Fig. 2) but did decrease thymidine incorporation within 12 hours. Thymidine incorporation in the presence of PTH decreased at 6 hours and increased at 12, 24, and 48 hours; however, these effects were not statistically significant. A23187 also decreased amino acid incorporation, but significant inhibition was not reached until 48 hours (data not shown). Histologic examination of bones cultured for 48 hours further reflected the difference between ionophores and PTH (Fig. 3). Control and PTH-treated bones showed abundant cellularity in the marrow cavity, whereas bones treated with ionophores showed marked loss of all cellular elements except for osteoclasts, which were increased. As they increased resorption, PTH, A23187, and Ionomycin also decreased the quantity of mineralized matrix present in the sections.

These results confirm that calcium



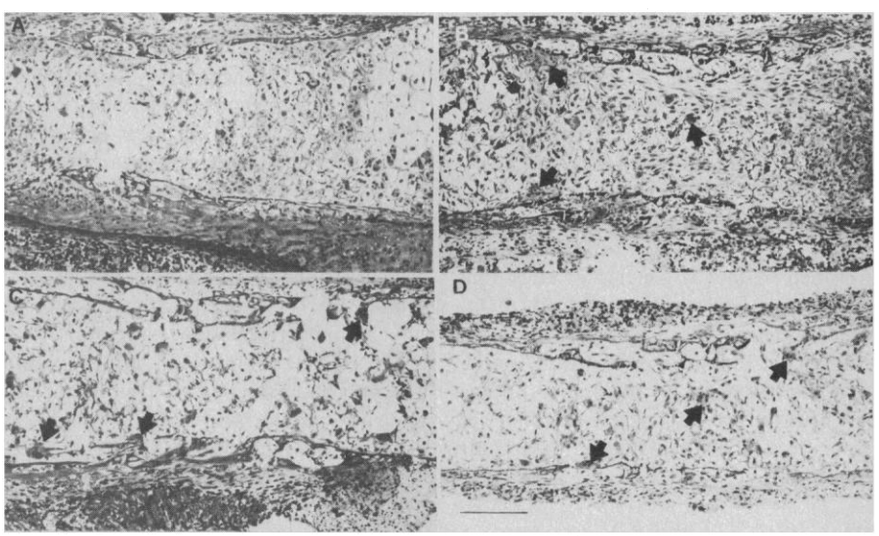


Fig. 2 (left). Comparison of the effects of PTH (0.04 μ*M*) and A23187 (0.3 μ*M*) on (A) bone resorption and (B) [^{3}H]thymidine incorporation at various times. In (A), ^{45}Ca release is significantly different from control ($P < .01$) for PTH (■) at 24 and 48 hours and for A23187 (□) only at 48 hours. In (B), [^{3}H]thymidine incorporation into bone is not significantly altered by PTH (●) at any time, whereas with A23187 (○) it is decreased significantly ($P < .01$) at 12, 24, and 48 hours. Values are means ± standard error for 12 to 20 determinations. Fig. 3 (right). Histologic sections of bones cultured for 48 hours with (A) control medium, (B) PTH, 0.04 μ*M*, (C) A23187, 0.3 μ*M*, and (D) Ionomycin, 1.0 μ*M*. Arrows point to osteoclasts. Scale bar, 100 μm.

ionophores can increase resorption in fetal rat long bones cultured in vitro. We believe this resorption, like that produced by PTH, is osteoclast mediated, since PTH, A23187, and Ionomycin all increase formation of multinucleated giant cells in bone and increase release of β-glucuronidase, an enzyme produced by activated osteoclasts (*9*). However, there are important differences between the action of calcium ionophores and PTH. For example, PTH had small effects on thymidine incorporation, whereas A23187 and Ionomycin markedly inhibited DNA synthesis. In addition, unlike PTH, both ionophores did not stimulate bone resorption at high concentrations. The effects of the ionophores on thymidine incorporation are similar to those observed by Dietrich and Paddock (*2*) in rat calvaria but differ from the effect of A23187 on lymphocyte cultures where DNA synthesis is increased (*10*).

The effects of high concentrations of ionophores on DNA and protein synthesis in bone could explain the inhibition of resorption observed by Dziak and Stern (*1*) and ourselves, as well as the inhibitory effects found by Ivey *et al.* (*3*) in mouse calvaria. Alternatively, these inhibitory effects on resorption could be due to a toxic action of the ionophores on mitochondrial function or adenosine triphosphate production (*11*). Feldman *et al.* (*12*) have recently shown that, in response to PTH, cultures of newborn mouse calvaria incorporate preexisting mononuclear precursors into functioning osteoclasts. Our results suggest that in cultures of fetal rat long bone, functioning osteoclasts can form without DNA synthesis and that divalent cation ionophores, like PTH, activate osteoclasts by stimulating precursor fusion through an effect which is probably mediated by an increase in intracellular calcium. The absence of any stimulatory effect on resorption by calcium ionophores in mouse calvaria could represent a species or tissue-specific difference from the rat long bone in the mechanism of osteoclast activation, particularly if mouse calvaria cultures were more dependent on cell replication for measurable osteoclastic bone resorption to occur.

Although our data do not rule out the possibility that ionophores cause an early initial stimulation of osteoclast precursor cell multiplication before inhibiting DNA synthesis, we expected that such an effect would also stimulate resorption in mouse calvaria. Moreover, in lymphocytes the mitogenic response produced by A23187 is not seen for 24 to 48 hours after treatment (*10*). It is also possible that multiplication of only a selected population of osteoclast precursors is necessary for resorption and that these cells are resistant to the inhibitory effects of calcium ionophores; however, this hypothesis seems unlikely because A23187 inhibited thymidine incorporation within 24 hours but did not produce resorption until 48 hours. At this time, A23187 at 1.0 μ*M* could still stimulate resorption, yet had reduced thymidine incorporation below our limit of detection. Thus, our results call into question the concept that cell replication is important for the stimulation of osteoclastic bone resorption by hormones, at least for fetal rat long bones. Not only did stimulation of resorption occur when DNA synthesis was inhibited by ionophores, but PTH had little stimulatory effect on thymidine incorporation in this system. We have also observed that the ability of PTH to stimulate bone resorption is not diminished in the presence of other inhibitors of DNA synthesis (*13*).

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DNA Sequence of Two Closely Linked Human Leukocyte Interferon Genes

Abstract. *A single recombinant lambda bacteriophage isolated from a human genome library contains two closely related human interferon genes of the leukocyte or α type. The two genes are separated by 12 kilobase pairs and are oriented in the same direction with respect to transcription. Comparisons of the DNA sequences of these two genes and interferon complementary DNA clones indicate that the two interferon genes lack intervening sequences.*

The human interferon multigene family consists of approximately ten leukocyte interferon (LeIF, IFN-α) genes (*1–3*) that have approximately 85 to 95 percent DNA sequence homology in their protein coding regions, and at least one fibroblast interferon (FIF, IFN-β) gene that is only about 50 percent homologous with the LeIF genes (*4–6*). The study of related gene families allows comparative analysis of nucleotide and amino acid sequences, which can help to identify features of importance for gene expression and protein function and can help elucidate modes of evolution of related genes. We therefore have determined the nucleotide sequence of eight distinct LeIF complementary DNA (cDNA) clones (*1*) and initiated DNA sequence analysis of LeIF genes isolated from a library of bacteriophage lambda clones containing overlapping fragments of the human genome. We now report the DNA sequences of two linked LeIF genes.

The phage λ Charon 4A recombinant library of the human genome constructed by Lawn *et al.* (*7*) was screened for leukocyte interferon genes (*7, 8*) with a radioactive probe derived from the cDNA clone LeIF A (*9*). Restriction endonuclease digestion and hybridization by the blot technique revealed that the recombinant designated λHLeIF2 contains an insert of 19 kilobase pairs (kb) of human DNA which, upon digestion with either of the restriction endonucleases Eco RI, Xba I, or Hind III, produces two fragments that hybridize to LeIF cDNA sequences. Subsequent analysis revealed two distinct LeIF genes in this 19-kb segment of the human genome (see below). A map indicating the location of the cleavage sites of several restriction endonucleases is pre-

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