JOURNAL OF CELLULAR PHYSIOLOGY 129:283–288 (1986)

Osteoclasts and Osteoblasts Migrate in Opposite Directions in Response to a Constant Electrical Field

JACK FERRIER,* STEPHEN M. ROSS, JUNYA KANEHISA, AND JANE E. AUBIN
Medical Research Council Group in Periodontal Physiology, University of Toronto, Toronto, Ontario, M5S 1A8 Canada

We have investigated in vitro the effects of the electrical field produced by constant current on freshly isolated rabbit osteoclasts and on well characterized clonal rat osteoblastlike cells. At field strengths of 0.1 and 1 V/mm, the osteoclasts migrated rapidly toward the positive electrode, whereas the osteoblastlike cells migrated in the opposite direction, toward the negative electrode. Thus, different cell types from the same tissue can respond differently to the same electrical signal. These results have important implications for hypotheses concerning the cellular mechanism of galvanotaxis, and may also clarify the cellular basis of the clinical application of electrical stimulation of bone healing.

The investigation of the effects of applied electrical fields on cells and tissues has expanded in recent years (Robinson, 1985). Such studies range from effects on the growth of neurites in culture (Jaffe and Poo, 1979; Hinkle et al., 1981; Patel and Poo, 1982) to clinical applications in bone healing (e.g., Bassett, 1984). Another area that has received considerable attention is that of the effect of the electrical field on cellular migration ("galvanotaxis"). Recent studies have involved work on embryonic fibroblasts (Erickson and Nuccitelli, 1984), fish epidermal cells (Cooper and Schliwa, 1985), and amphibian neural crest cells (Stump and Robinson, 1983; Cooper and Keller, 1984). All these cell types have been reported to show galvanotaxis directed toward the negative electrode (cathode). Some experiments have been carried out at field strengths as low as 0.01 V/mm (Stump and Robinson, 1983; Erickson and Nuccitelli, 1984), but most were in the range of 0.1–1.5 V/mm. We have investigated the effect of the electrical field produced by constant current at field strengths of 0.1 and 1 V/mm on the migration of freshly isolated mammalian osteoclasts and on well characterized clonal osteoblastlike cells.

MATERIALS AND METHODS

Osteoclasts were obtained by explantation directly from humeri, femurs, and tibiae of newborn rabbits according to the method of Chambers et al. (1984). They were confirmed to be able to make resorption lacunae on bone slices in vitro (J. Kanehisa, and J.N.M. Heersche, submitted) and to contract in response to calcitonin. Two osteoblastlike lines, RCJ 1.20 and RCB 2.2A, were derived from fetal rat calvaria cell populations by limiting dilution cloning and have been characterized in terms of their collagen production and cAMP response to hormones (Aubin et al., 1982; Heersche et al., 1985). The populations from which these lines were derived have been shown to have the capability of forming bone in culture (Bellows et al., 1986). The electrophysiological properties of the osteoclasts and the osteoblastlike lines used in this study have also been studied (Ferrier et al., 1982, 1985, 1986; Dixon et al., 1984; Ferrier and Ward, 1986).

Thin chambers of cross section 150 μm × 10 mm were constructed (Jaffe and Poo, 1979; Patel and Poo, 1982; Erickson and Nuccitelli, 1984; Cooper and Schliwa, 1985). The chambers were 40 mm long. Two strips of No. 1 glass coverslip were placed 10 mm apart on the bottom of a 150-mm tissue culture dish, and a third coverslip was placed on top. Wells were formed at both ends of this chamber by means of glass barriers, and the whole assembly was sealed along the edges by means of stopcock grease. To avoid contamination with electrode products, 500-mm salt bridges filled with α-minimum essential medium (α-MEM) in 3% agar were used to connect the chamber wells to flasks containing Ag/AgCl electrodes. The entire chamber was sterilized in a γ-irradiator before plating of cells in α-MEM buffered to pH 7.3 with 25 mM HEPES and supplemented with 15% fetal bovine serum and antibiotics. After cells had attached and spread, the chamber was placed on the stage of a phase-contrast inverted microscope (Nikon Diaphot) for time lapse photography on 16-mm cine film. A graduated scale placed in a culture dish was filmed to provide a calibration scale for distance. The microscope had a temperature-controlled housing to keep the chambers at 37°C during the experiments.

For the osteoclast experiments, there were one to four osteoclasts in the field of view of the cine camera. The velocity of each was measured by tracing the cellular position at different times from the projection of the cine film. For experiments with osteoblastlike cells, there were from 10 to 200 cells in the field of view. The velocity of individual osteoblastlike cells was measured by tracing cellular positions at different times for at least 10 representative cells from each experiment. All mean values are reported as mean ± SEM.

Received March 14, 1986; accepted July 21, 1986.
*To whom reprint requests/correspondence should be addressed.

RESULTS

At an electrical potential gradient of 1 V/mm, with a lag time following application of the field of from 5 to 45 min, the osteoblastlike cells displayed prominent lamellipodia on their cathodal sides and cellular migration directed toward the cathode (Fig. 1). None of these cells migrated toward the positive electrode (anode) at this field strength. These experiments were carried out with cultures that were nearing confluence. Although this condition is closer to the in vivo condition than a very sparse culture would be, migration speeds may have been reduced in some cases because of contact inhibition. In three experiments with RCJ 1.20 cells, and two experiments with RCB 2.2A cells, the individual cellular speeds ranged between 5 and 32 μm/hr. When the field direction was reversed, there was a reversal of direction of migration, with a lag time of from 0.5 to 2.0 hr.

At an electrical potential gradient of 0.1 V/mm, the osteoblastlike cells showed a considerable lag time before responding to the field. In one experiment with RCJ 1.20 cells, the centers of all cells were plotted at the beginning, the midpoint, and the end of a 17.2 hr exposure to the field as shown in Figure 2. During the first 8.6 hr., 13 cells had clearly moved toward the cathode, 10 toward the anode, and six others were within 10° of moving perpendicularly to the potential gradient. There was thus no significant directed migration during this period. During the second 8.6 hr period, 35 cells moved toward the cathode, nine toward the anode, and three perpendicularly to the field vector. Even if we count the perpendicularly moving cells with the anodal ones, the binomial theorem and the χ^2 test both show the probability of this result being a chance occurrence to be less than 0.001. The mean cellular velocity component in the direction of the field vector (averaged over all cells) was 3.01 $\pm$ 0.55 μm/hr, which is significantly different from zero using the t test at $P < 0.001$.

In another experiment at 0.1 V/mm with RCJ 1.20 cells, after 1 hr of field exposure, three of 10 cells in the field of view were clearly migrating toward the cathode; the other seven cells appeared to be randomly motile. After 3.2 hr, eight of the 10 cells were clearly migrating toward the cathode. This has a probability of being a chance occurrence of less than 0.06. The mean velocity in the direction of the field vector was 20.3 $\pm$ 5.7 μm/hr, which is significantly different from zero at $P < 0.01$. In an experiment at 0.1 V/mm with RCB 2.2A cells, following the first 3.1 hr of field exposure, seven cells had moved toward the cathode, two toward the anode, two perpendicularly, and twenty had not moved. There was thus no significant directed migration. During the next 3.8 hr, 14 cells moved toward the cathode, three toward the anode, one perpendicularly, and 11 were not motile. This has a probabiity of being a chance occurrence of less than 0.02. The mean velocity in the direction of the field vector was 2.7 $\pm$ 1.0 μm/hr, which is significantly different from zero at $P < 0.02$. In this experiment, when the field strength was increased to 1 V/mm, there was a maximum lag time of 1.5 hr, after which all the cells clearly migrated toward the cathode. This persisted for 6 hr.

At a field strength of 1 V/mm, with a time lag of from 2 to 10 min, all the osteoclasts showed oriented lamellipodia and directed migration toward the positive electrode. The mean speed of migration for 20 cells from seven experiments was 138 $\pm$ 16 μm/hr. The mean value of the cosine of the angle between the potential gradient and the cellular velocity was 0.96 $\pm$ 0.01. The mean value of the component of velocity in the direction of the anode was 132 $\pm$ 15 μm/hr. Upon reversal of the field,

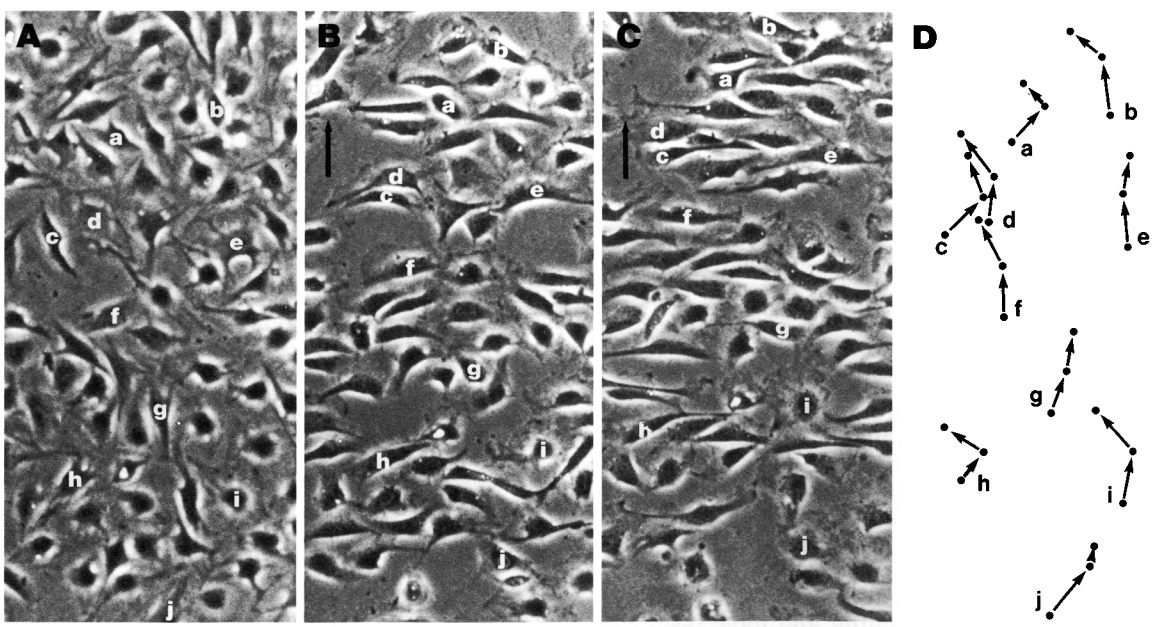

Fig. 1. Osteoblastlike RCJ 1.20 cells. A, just before current was applied; B, in current, 2 hr after current was first applied; and C, in current, 4 hr after current was first applied. The arrow shows the direction of the uniform electrical current (negative electrode at top), with an electrical potential gradient of 1 V/mm. The length of the arrow indicates 50 μm. D, the movement of the labeled cells. The identity of the labeled cells was verified by following them on the time-lapse film. Note the prominent lamellipodia directed toward the cathode.

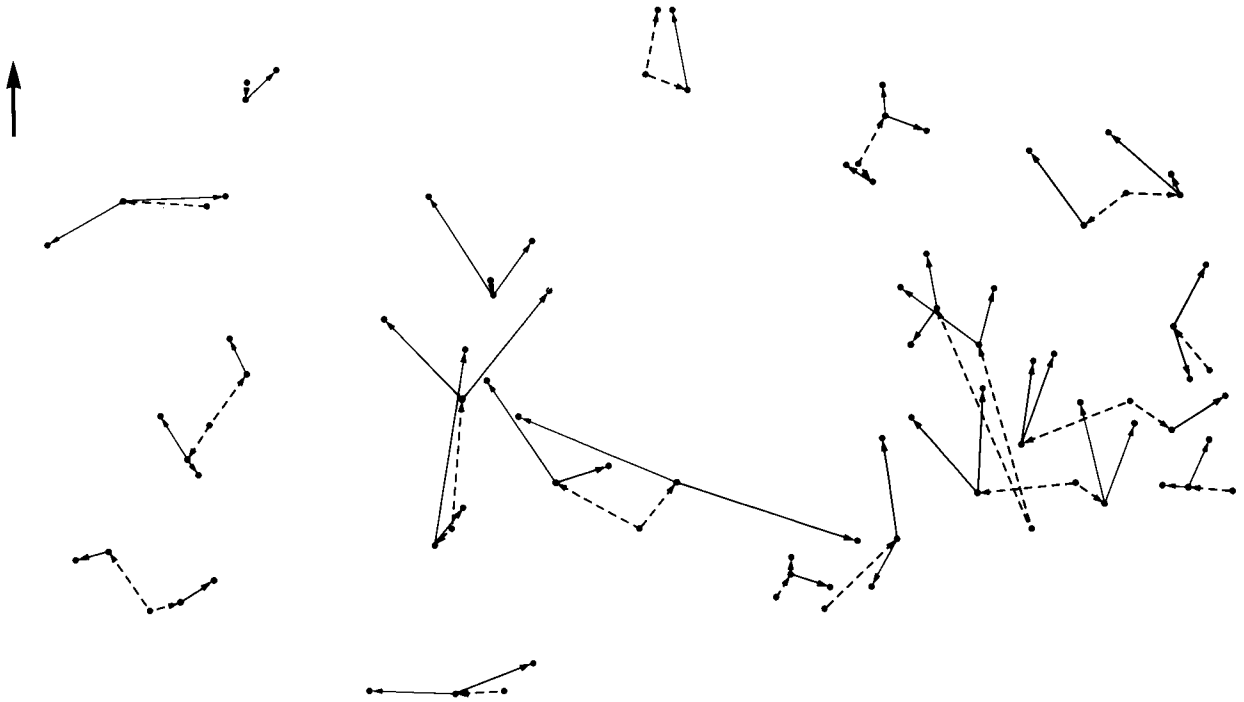

Fig. 2. Osteoblastlike RCJ 1.20 cell centers at the beginning, midpoint, and end of a 17.2 hr, 0.1 V/mm field exposure. All cells in the field of view of the time-lapse camera were included in the diagram. The dashed arrows show the movement of the cell centers during the first 8.6 hr period. The solid arrows show the movement of the cell centers during the second 8.6 hr period. The diagram has a "cascade" appearance because the cells are proliferating with a doubling time of 14.2 hr, which is normal for this cell line. The heavy arrow shows the direction of the uniform electrical current. The length of this arrow indicates 50 μm.

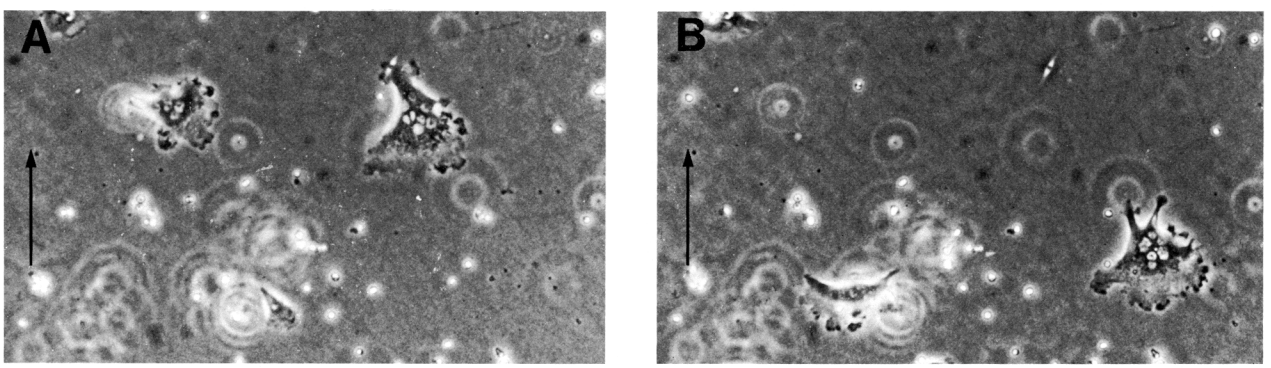

Fig. 3. Osteoclasts in the applied current. A, 1.8 hr after current was first applied; B, 4.4 hr after current was first applied. The arrow shows the direction of the uniform electrical current, with a 0.1 V/mm electrical potential gradient. The length of the arrow indicates 100 μm.

the migration direction reversed with a lag of from 3 to 10 min. Directed migration was observed for as long as 10 hr in some experiments, as cells entered and moved across the field of view. Before field application, the osteoclasts showed random motility with a much lower speed.

At a field strength of 0.1 V/mm, with a lag time of from 1.0 to 2.7 hr, the osteoclasts showed oriented lamellipodia and clearly migrated toward the anode (Fig. 3). The mean speed for 10 cells from three experiments was 72 $\pm$ 13 μm/hr. The mean value of the cosine of the angle between the potential gradient and the cellular velocity was 0.97 $\pm$ 0.01. The mean value of the component of velocity in the direction of the anode was 70 $\pm$ 12 μm/hr. Upon increasing the field strength to 1 V/mm, the osteoclasts increased their speed of migration, with a lag of from 1 to 2 min.

The time period for measurement of velocity in the electrical field was variable because of the different period of time that each cell remained in the field of view. The time period of the measurement (in hours), the cosine of the angle between the potential gradient and the velocity, and the component of velocity in the direction of the anode (μm/hr), for each of the 20 osteoclasts at 1 V/mm, were: 1.6, 0.999, 158; 1.6, 0.946, 70; 2.0, 0.998, 110; 2.0, 0.995, 202; 2.0, 0.966, 119; 2.0, 0.999,

266; 2.0, 0.996, 119; 2.7, 0.857, 147; 1.5, 1.000, 182; 1.5, 0.970, 175; 1.0, 0.917, 34; 2.0, 0.983, 237; 0.6, 0.839, 144; 0.5, 0.829, 216; 0.7, 0.989, 101; 0.9, 1.000, 29; 0.6, 0.930, 65; 0.4, 0.986, 156; 2.8, 0.994, 57; 4.0, 0.997, 50. For each of the 10 osteoclasts at 0.1 V/mm, these values were: 3.5, 0.875, 13; 3.5, 0.985, 19; 3.2, 0.998, 66; 3.2, 0.914, 46; 4.7, 0.914, 46; 1.8, 0.966, 94; 1.9, 1.000, 105; 5.4, 0.933, 79; 4.0, 0.974, 130; 6.8, 0.983, 77. The velocity vectors for each of these osteoclasts are shown in Figure 4.

DISCUSSION

The main result of our experiments is that osteoclasts migrate in an applied current toward the anode. It is interesting to note that early studies on unfractionated leucocytes showed that these cells displayed galvanotaxis toward the anode (Monguio, 1933; Fukushima et al., 1953). Orida and Feldman (1982) have shown that macrophages extend lamellipodia toward the anode in applied current experiments. An accumulating body of evidence supports the hypothesis that osteoclasts derive from a hematopoietic precursor, i.e., a bone marrow mononuclear cell, possibly a cell of the monocyte-macrophage series (Gothlin and Erickson, 1973; Kahn and Simmons, 1975; Teitelbaum and Kahn, 1980; Owen, 1980; Bonucci, 1981; Marks and Walker, 1981). On the other hand, osteoblasts are thought to arise from undifferentiated mesenchymal cells that are components of the stromal connective tissue (Friedenstein, 1976; Owen, 1980). Thus these results show that cells of hematopoietic descent exhibit anodal galvanotaxis, whereas osteoblasts and a wide variety of other cell types display cathodal galvanotaxis (Stump and Robinson, 1983; Cooper and Keller, 1984; Erickson and Nuccitelli, 1984; Cooper and Schliwa, 1985).

These findings point toward a mechanism that may be involved in natural remodelling and healing of bone, and in clinical applications of electrical stimulation of bone healing. Regions of high bone formation activity have been reported to have a negative extracellular electrical potential relative to less active regions (Rubinacci and Tessari, 1983). This effect might be enhanced by the application of exogenous current in the case of direct current healing of bone nonunions, in which the cathode is inserted into the nonunion (Bassett, 1984).

Hypotheses concerning the mechanism of galvanotaxis have been developed (Stump and Robinson, 1983; Erickson and Nuccitelli, 1984; Cooper and Keller, 1984; Cooper and Schliwa, 1985; Robinson, 1985). Some of these involve the idea that lamellipodia extension and cellular migration in the applied field occur toward the side of the cell experiencing a Ca^{2+} influx. However, in light of the results presented in this paper, this hypothesis could be valid only if the osteoclast Ca^{2+} channels have a different voltage dependence than those in osteoblasts. The potential difference across the cell membrane facing the cathode will be depolarized relative to the membrane potential before the current is applied, whereas the cell membrane facing the anode will be hyperpolarized. If the osteoblast Ca^{2+} channels have an increased probability of being open when the membrane poential is depolarized, which is assumed to be a normal behavior for such channels, then there will be an influx of Ca^{2+} on the cathodal side of the cell. On the other hand, the osteoclast Ca^{2+} channels would be required to have an increased probability of being open when the membrane potential is hyperpolarized, but not when depolarized, to have a significant Ca^{2+} influx on the anodal side of the cell but not on the cathodal side of the cell. The change in inward driving force for Ca^{2+} would be too small to have a significant effect.

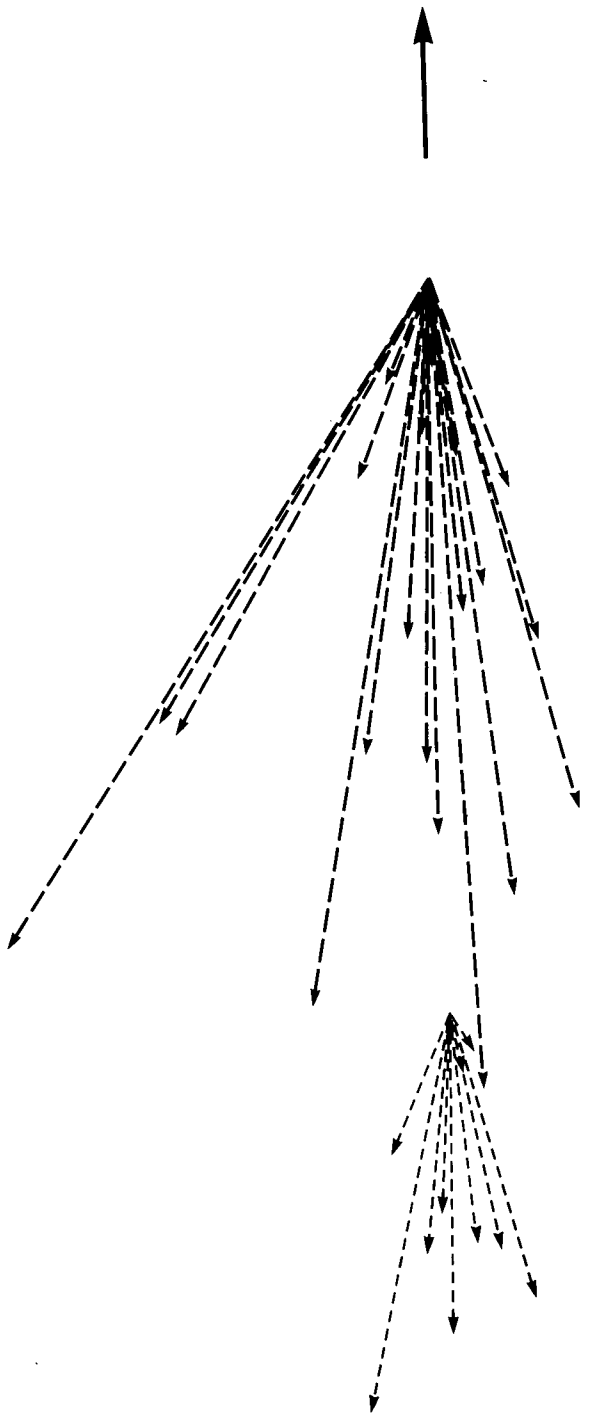

Fig. 4. Velocity vectors for osteoclasts in an applied electrical field. The heavier dashed vectors represent the velocities at 1 V/mm. The lighter dashed vectors represent the velocities at 0.1 V/mm. The solid arrow shows the direction of the uniform electrical current. The length of this arrow indicates 50 μm/hr.

However, the effect of K^+ and Na^+ transport should be also considered. It has been shown that osteoblastlike

cells have Ca^{2+}-activated K^+ channels in the cell membrane (Ferrier et al., 1982, 1985; Dixon et al., 1984; Ferrier and Ward, 1986). An influx of Ca^{2+} on the depolarized cathodal side of the osteoblastlike cell should result in activation of these K^+ channels. This would tend to repolarize the cathodal side of the cell, thus reducing the Ca^{2+} conductance there. This effect, as well as the response of the Ca^{2+} pump to an increased internal $[Ca^{2+}]$, would bring internal $[Ca^{2+}]$ back toward the unperturbed level. This, in turn, would tend to reduce K^+ conductance to its unperturbed level, which would allow the entire course of events to repeat. It is possible that such a repeated course of net Ca^{2+} influx and efflux on the cathodal side of the osteoblastlike cell could result in the observed motile activity on that side of the cell.

On the other hand, the osteoclast has been shown to have a higher specific membrane resistance and to have a membrane potential that is less sensitive to extracellular $[Ca^{2+}]$ than the osteoblastlike cell (Ferrier et al., 1986). This suggests that the osteoclast cell membrane has fewer Ca^{2+}-activated K^+ channels and fewer Ca^{2+} channels, so that the hypothesis outlined above would not apply to the osteoclast. Another mechanism that can lead to an increase in internal $[Ca^{2+}]$ is that of a change in Na^+/Ca^{2+} exchange across the cell membrane following an increase in internal $[Na^+]$ (see, e.g., Lee et al., 1985). Thus one hypothesis for a mechanism of osteoclast galvanotaxisis would be that the net Na^+ influx on the anodal side of the cell (through Na^+ channels), produced by the hyperpolarization of that side, would result in a net Ca^{2+} influx via an altered Na^+/Ca^{2+} exchange rate and that this would lead to motile activity on that side of the cell. Hypotheses such as these can be tested using the microelectrode and patch clamp techniques.

Another class of hypotheses that has been proposed involves the electrophoresis or electroosmotically induced movement of membrane proteins (Stump and Robinson, 1983; Erickson and Nuccitelli, 1984; Cooper and Keller, 1984; Cooper and Shliwa, 1985; Robinson, 1985). The lag time for initial response to the field that was observed at 0.1 V/mm and the lag time for osteoblastlike cells to respond to a reversed field are comparable to the lag times reported by Stump and Robinson (1983) for neural crest cells. The existence of such long lag times supports the possibility that electrophoresis or electroosmosis is involved in the mechanism of response, since these processes are inherently much slower than the openning of voltage-dependent ion transport channels. There is no upper limit to the time required for electrophoresis or electroosmotically induced movement of membrane proteins, because such proteins can have varying degrees of mobility within the cell membrane (see, e.g., Almers and Stirling, 1984). Differences in such mobilities between different cell types provide another possibility for differences in response to an applied electrical field.

The applied electrical field appeared to have no adverse effects on the cells. Both the osteoclasts and the osteoblastlike cells maintained a normal, well spread morphology in the applied field, with no increase in intracellular granularity (e.g., Figs. 1 and 3). A number of osteoblastlike cells were observed to go through at least one cell cycle with a normal generation time (Fig. 2). This indicates that directed migration induced by an electrical field does not interfere with the cells' ability to proliferate. Although the osteoblastlike cells, and some of the osteoclasts, migrated with their long axes perpendicular to the direction of movement, which is different from the nonelectrically induced case, the leading lamel-lipodia and the retraction fibers on the trailing sides were similar to those seen in nonelectrically induced migration. Thus electrically induced cellular migration may be essentially the same as migration induced by other signals.

ACKNOWLEDGMENTS

We thank Mr. Alan Illeman for providing excellent technical assistance and Prof. Johan Heersche for advice and guidance to J.K. while he was perfecting the method for obtaining and maintaining osteoclasts.

LITERATURE CITED

Aubin, J.E., Heersche, J.N.M., Merrilees, M.J., and Sodek, J. (1982) Isolation of bone cell clones with differences in growth, hormone response, and extracellular matrix production. J. Cell Biol., *92*:452–461.

Almars, W., and Stirling, C. (1984) Distribution of transport proteins over animal cell membranes. J. Membrane Biol., *77*:169–186.

Bassett, C.A.L. (1984) The electrical management of ununited fractures. In: Complications of Fracture Management. H.R. Gossling and S.L. Pillsbury, eds. Lippincott, Philadelphia, pp. 9–39.

Bellows, C.G., Aubin, J.E., Heersche, J.N.M., and Antosz, M.E. (1986) Mineralized bone nodules formed in vitro from enzymatically released rat calvaria cell populations. Calcif. Tissue Int., *38*:143–154.

Bonucci, E. (1981) New knowledge on the origin, function and fate of osteoclasts. Clin. Orthop. Rel. Res., *158*:252–269.

Chambers, T.J., Revell, P.A., Fuller, K., and Athanason, N.A. (1984) Resorption of bone by isolated rabbit osteoclasts. J. Cell Sci., *66*:383–399.

Cooper, M.S., and Keller, R.E. (1984) Perpendicular orientation and directional migration of amphibian neural crest cells in dc electrical fields. Proc. Natl. Acad. Sci. USA, *81*:160–164.

Cooper, M.S., and Schliwa, M. (1985) Electrical and ionic controls of tissue cell locomotion in dc electric fields. J. Neurosci. Res., *13*:223–244.

Dixon, S.J., Aubin, J.E., and Dainty, J. (1984) Electrophysiology of a clonal osteoblast-like cell line: Evidence for the existence of a Ca^{2+}-activated K^+ conductance. J. Membrane Biol., *80*:49–58.

Erickcson, C.A., and Nuccitelli, R. (1984) Embryonic fibroblast motility and orientation can be influenced by physiological electric fields. J. Cell Biol., *98*:296–307.

Ferrier, J., Dixon, J., Illeman, A., Dillon, E., and Smith, I. (1982) Low-frequency voltage noise in a mammalian bone cell clone. J. Cell. Physiol., *113*:267–272.

Ferrier, J., Illeman, A., and Zakshek, E. (1985) Transient and sustained effects of hormones and calcium on membranes potential in a bone cell clone. J. Cell. Physiol. *122*:53–58.

Ferrier, J., and Ward, A. (1986) Electrophysiological differences between bone cell clones: Membrane potential responses to parathyroid hormone and correlation with the cAMP response. J. Cell. Physiol. *126*:237–242.

Ferrier, J., Ward, A., Kanahisa, J., and Heersche, J.N.M. (1986) Electrophysiological responses of osteoclasts to hormones. J. Cell. Physiol., *128*:23–26.

Friedenstein, A.J. (1976) Precursor cells of mechanocytes. Int. Rev. cytol., *47*:327–359.

Fukushima, K., Senda, N., Inui, H., Miura, H., Tamai, Y., and Murakami, Y. (1953) Studies on the galvanotaxis of leukocytes. I. Galvanotaxis of human neutrophilic leukocytes and methods of its measurement. Med J. Osaka Univ., *41*:195–208.

Gothlin, G., and Erickson, J.L.E. (1973) On the histogensis of the cells in fracture callus. Virchows Arch. Abt. B Zellpathol., *12*:318–329.

Heersche, J.N.M., Aubin, J.E., Grigoriadis, A.E., and Moriya, Y. (1985) Hormone responsiveness of bone cell populations. In: The Chemistry and Biology of Mineralized Tissue. W.T. Butler, ed. Ebsco Media, Birmingham, Alabama, pp. 286–295.

Hinkle, L., McCraig, C.D., and Robinson, K.R. (1981) The direction and growth of differentiating neurons and myoblasts from frog embryos in an applied electric field. J. Physiol., *314*:121–135.

Jaffe, L.F., and Poo M.-M. (1979) Neurites grow faster towards the cathode than the anode in a steady field. J. Exp. Zool., *209:*115–128.

Kahn, A.J., and Simmons, D.J. (1975) Investigation of cell lingeage in bone using a chimaera of chick and quail embryonic tissue. Nature. *258:*325–327.

Lee, C.O., Abete, P., Pecker, M., Sonn, J.K., and Vassalle, M. (1985) Strophanthidin inotrophy: Role of intracellular sodium ion activity and sodium-calcium exchange. J. Mol. Cell. Cardiol., *17:*1043–1053.

Marks, S.C., and Walker, D.G. (1981) The hematogenous origin of osteoclasts: Experimental evidence from osteopetrotic (microphthalmic) mice treated with spleen cells from beige mouse donors. Am. J. Anat., *161:*1–10.

Monguio, J. (1933) Uber die polare wirkung des galvanischen stromes auf leukozyten. Z. Biol., *93:*553–559.

Orida, N., and Feldman, J.D. (1982) Directional protrusive pseudopodial activity and motility in macrophages induced by extracellular electric fields. Cell Motil., *2:*243–255.

Owen, M. (1980) The origin of bone cells in the postnatal organism. Arthritis Rheum., *23:*1073–1080.

Patel, N., and Poo, M.-M. (1982) Orientation of neurite growth by extracellular electric fields. J. Neurosci. *2:*483–496.

Robinson, K.R. (1985) The responses of cells to electrical fields: a review. J. Cell Biol., *101:*2023–2027.

Rubinacci, A., and Tessari, L. (1983) A correlation analysis between bone formation rate and bioelectric potentials in rabbit tibia. Calcif. Tissue Int., *35:*728–731.

Stump, R.F., and Robinson, K.R. (1983) Xenopus neural crest cell migration in an applied electrical field. J. Cell Biol., *97:*1226–1233.

Teitelbaum, S.L., and Kahn, A.J. (1980) Mononuclear phagocytes, osteoclasts and bone resorption. Mineral Electrolyte Metab., *3:*2–9.