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Low Frequency Electric Field Induced Changes in the Shape and Motility of Amoebas

Abstract. *Perpendicular and parallel elongation of the giant amoeba,* Chaos chaos (Chaos carolinensis), *have been observed in alternating electric fields over a wide frequency range (from about 1 hertz to about 10 megahertz). The characteristics change as a function of frequency. Simple dielectric forces may be important in the production of these effects.*

Amoeboid cells crawl parallel to a d-c electric field toward the cathode at only a few volts per centimeter (*1*). Mast reported that *Amoeba proteus* extended pseudopodia perpendicular to a 60-hz a-c electric field (*2*). Shape and motility changes in a-c electric fields were reported as early as the 1860's (*3, 4*), but with only limited data on frequency dependence. Recently, shape and orientation changes were reported in many small cells and inanimate particles subjected to radio-frequency electric fields at several hundred volts per centimeter and were attributed to dielectric field induced forces (*5*), including the elongation of erythrocytes (*6*).

We now describe shape, orientation, and motility changes in the giant amoeba, *Chaos chaos (Chaos carolinensis)*, over a wide range of frequencies (1 hz to 10 Mhz) and point out that the simple physical mechanism of dielectric forces may be important for low frequency as well as radio-frequency phenomena.

Chaos, because of its size (0.5 mm or more in length) was used, making handling and observation easier, and its dilute culture fluid—K_2HPO_4 (0.16 m*M*), KH_2PO_4 (0.11 m*M*), $MgSO_4$ (0.05 m*M*), and $Ca(NO_3)_2$ (0.50 m*M*) in glass-distilled water, conductivity 0.20 mmho/cm—reduced electrode polarization and heating. The *Chaos* (*7*) were cultured by standard methods (*8*) and starved for 1 day to eliminate food vacuoles. They were placed in the chamber at least 1 mm from each platinized platinum wire (0.1 mm diameter) electrode between parallel glass surfaces 0.2 mm apart, which had been freshly cleaned with nitric acid to enhance adhesion. Sudden increases, of 30 percent or more, in field intensity were avoided as they often carried past the desired event. Particles of three highly purified long-chain hydrocarbons—octadecane (m.p., 28° to 30°C), eicosane (m.p., 35° to 36.5°C), and docosane (m.p., 43° to 45°C)—indicated the temperature by melting point. The fluid never exceeded 30°C in most of the experiments; it rose above 30°C only where the field exceeded ~ 125 volt/cm.

The three photographs of a single *Chaos* at successive stages of perpendicular elongation (Fig. 1, a to c) were taken within 10 minutes at about 15 volt/cm (root-mean-square) and 316 hz. The character of the elongation changes (which are not rotational) vary with frequency in the following ways: (i) Below ~ 1 hz changes occur, but there is no orientation; (ii) between ~ 1 and ~ 100 hz, pseudopodia extend perpendicular to the field, but complete alignment never occurs; (iii) between ~ 100 hz and ~ 1 khz, parallel pseudopodia withdraw, and perpendicular pseudopodia extend to the extreme of Fig. 1c; (iv) between ~ 1 and ~ 100 khz, *Chaos* loses its pseudopodia and becomes approximately elliptical perpendicular to the field; and (v) above ~ 100 khz, *Chaos* remains elliptical (Fig. 1, d and e), but deforms with its long axis parallel to the field. The fluid circulates violently, and cellular material streams toward the electrodes. The cell is always destroyed.

Field strengths less than ~ 10 volt/cm produce these effects at frequencies below ~ 1 khz. Above ~ 1 khz the required field strength steadily increases. At ~ 100 khz it may exceed 200 volt/cm.

The character of the effects is also a function of field strength. The first observable effect (at a few volts per centimeter) in the middle range of frequencies (~ 10 hz to ~ 1 khz) is an inward drift of the cytoplasm parallel to the field, which the amoeba can overcome up to ~ 10 volt/cm. The ratio of sol to gel does not appear to change. Above ~ 10 volt/cm the perpendicular form shown in Fig. 1, b and c, occurs. Often one pseudopodium forms, rather than the two oppositely directed pseudopodia shown.

Perpendicular elongation up to ~ 15 volt/cm can be repeated many times without apparent damage to the amoeba. The *Chaos* shown in Fig. 1, a to c, experienced elongation twice during the preceding half hour, but was indistinguishable from a normal amoeba within 5 to 10 minutes after the field was removed. Above ~ 15 volt/cm in the middle range of frequencies (~ 10 hz to ~ 1 khz) the ratio of sol to gel increases, the contractile vacuoles swell, sudden internal motions of the cytoplasm occur, the clear hyaline layer beneath the membrane disappears, and disturbances of the membrane occur—effects that are well known (*1*).

Above ~ 25 volt/cm, at radio frequency (that is, 0.5 to 10 Mhz), the amoeba becomes rigid, the ratio of sol to gel apparently shifted to gel; there is no preferential orientation. A similar effect was noted by Kühne, using an induction coil and human leukocytes (*4*). Parallel orientation finally occurs when the field exceeds ~ 200 volt/cm.

Two principal theories of amoeboid motion are the "fountain-zone-contraction" model of Allen (*9*) and the "contraction-hydraulic" model of Jahn and Bovee (*10*). Jahn suggests that d-c galvanotaxis is caused by calcium ions collecting on the anodal side because of the ion exchange properties of the cellular materials, and resulting in localized contraction (*11*). Referring to Helfferich (*12*), Jahn also suggests that a similar cation-sorting process may occur in a-c electric fields.

Dielectric forces are another important factor in the a-c case. Oscillating electric fields induce simple physical forces in structures composed of differing dielectric materials (*13*). Dielectric force effects are well described and have been investigated both theoretically and experimentally (*14*); they include "pearl chain formation" (*5, 13*), "dielectrophoresis" (*13, 15*), and "orientation" (*5, 6, 13*). Underlying these effects is the tendency of the system to minimize its potential energy. Parallel elongation above ~ 100 khz (Fig. 1e) is almost certainly due to such forces; the violent circulation of the fluid and material shooting toward the electrodes can have nothing to do with any cellular process. There is a transition into the apparently normal but externally controlled perpendicular motion of the amoeba at lower frequencies (< 1 khz), with no distinct break. At 100 khz some *Chaos* are parallel, others are perpendicular.

"Pearl chain" formation and "orientation" occur in *Escherichia coli* at several hundred volts per centimeter; the square of the threshold field strength is proportional to the inverse of the volume of the particle under study (*13*). By extrapolation, if *E. coli* (1 μm) were increased to the size of *Chaos* (500 μm), they would be oriented even at field intensities an order of magnitude lower than those required to orient *Chaos*. Therefore, objects with the size and general dielectric properties of *Chaos* should experience significant dielectric forces in the range of field strengths where low frequency perpendicular elongation occurs. The passive or active resistance of *Chaos* could increase the required forces. The considerable increase in threshold field strength with increasing frequency leading to other shapes and orientations is consistent with a dielectric force mechanism, since the induced dipole moment and potential energy of the *Chaos* cell decreases rapidly above ~ 1 khz, as may be seen from an appropriate application of Laplace field theory to cells (*14, 16*).

In conclusion, the perpendicular extension observed by Mast in *Amoeba proteus* at 60 hz has been demonstrated in *Chaos* over a wide range of frequencies from 1 hz to 100 khz. Perpendicular extension changes into parallel elongation above 100 khz. Dielectric forces may play an important role in the range of 1 hz to 100 khz.

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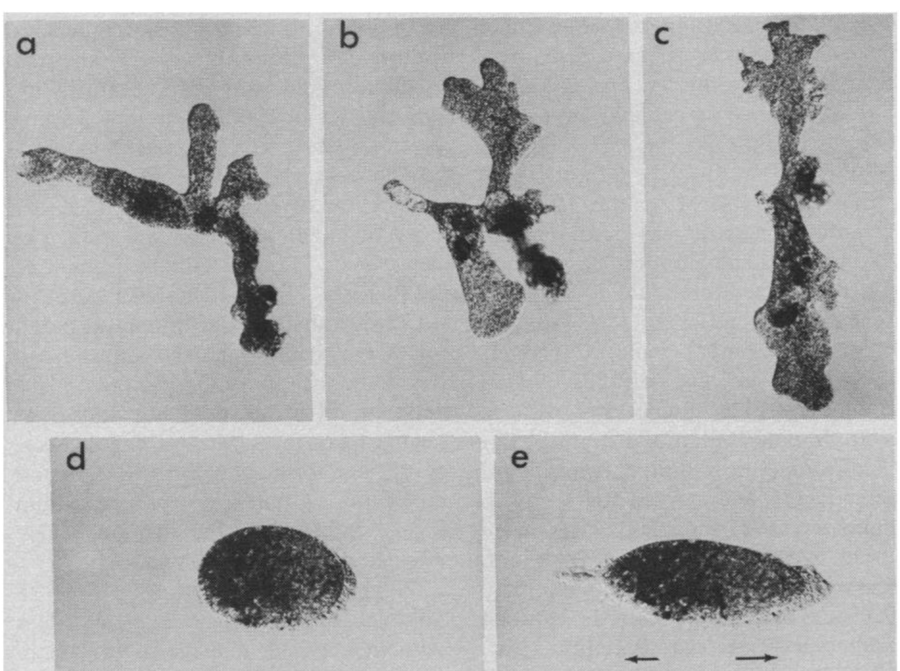

Fig. 1. Deformation of *Chaos* in an electric field. (a) Normal *Chaos* moving freely in the absence of an electric field. (b and c) Successive photographs of the same *Chaos* withdrawing its parallel pseudopodia and extending perpendicular pseudopodia in an a-c electric field at about 15 volt/cm and 316 hz. The whole sequence from (a) to (c) took place in about 10 minutes. This same *Chaos* had already been elongated twice during the preceding half hour without any apparent damage or change in its morphology. (d and e) Successive photographs of a different *Chaos* exposed to an electric field at 250 volt/cm and 5 Mhz. The amoeba elongated parallel to the field and was destroyed. A violent circulation of the surrounding culture fluid occurred, and material ultimately streamed off the amoeba toward the electrodes. The bar at the lower left indicates 0.5 mm, and the arrows at the lower right indicate field direction.

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Growth, Behavior, and Brain Catecholamines in Lead-Exposed Neonatal Rats: A Reappraisal

Abstract. *Daily oral administration of lead to newborn rats has no adverse effect on their body growth. Lead-treated rats were more active than age-matched controls. Endogenous levels of brain dopamine were unchanged, whereas norepinephrine was increased, suggesting a possible relationship between lead exposure during earliest developmental periods, increased motor activity, and brain norepinephrine, and not brain dopamine as previously postulated.*

Neurological manifestations of lead poisoning can be induced in suckling rats by feeding a diet containing 4.5 percent lead carbonate to the lactating mother rats. Lead is transmitted to the young via the maternal milk (*1, 2*). There is a pronounced retardation in growth rate in sucklings from lead-exposed mothers, and during the fourth week they develop paraplegia and extensive reddish-brown pigmentation of the cerebellum (*1–3*).

A lactating mother rat eating 5 percent lead acetate (2.73 percent lead) produces milk containing 25 parts of lead per million (ppm) (*4*). When the mother's diet is changed just prior to weaning from 5 percent lead acetate to one containing 25 ppm lead, and neonates are allowed free access to the same solid maternal diet, the sucklings still have retarded body growth but do not develop paraplegia or grossly apparent vascular damage of the cerebellum. However, during the fourth week these animals exhibit hyperactivity, tremors, and stereotyped behavior (*4–6*). It has also been reported that such behavior was manifest at a time when there is an eightfold increase in the concentration of lead in brain, and no apparent change in norepinephrine but a 20 percent decrease in dopamine relative to coetaneous controls (*6*).

The fact that the young, even after weaning to the lower lead diet, still have retarded body growth confounds the study by adding the factor of possible undernutrition to such an investigation.

We now report that the daily oral administration of lead acetate solution (1.09 mg of lead) to suckling rats does not influence growth relative to coetaneous controls and that lead-exposed animals show periods of increased motor activity. Unlike Sauerhoff and Michaelson, in their earlier report (*6*) from this laboratory, we are unable to detect a statistically significant lowering of brain dopamine levels, but we do find slight increases in norepinephrine.

The rats used in our study were timed pregnant Sprague-Dawley rats obtained from ARS/Sprague-Dawley Co., Madison, Wisconsin, and their offspring. The rats were housed as previously described (*4–6*). They were then divided into three groups, as follows:

Group A (controls): nursing mothers consuming normal powdered laboratory chow and tap water, and sucklings given daily oral doses of 0.1 ml of 2 percent sodium acetate and weaned on day 16 to normal diets;

Group B: nursing mothers consuming normal powdered laboratory chow and tap water, and sucklings receiving daily oral doses of 2 percent lead acetate (0.1 ml, 1.0 mg of lead) and weaned on day 16 to a diet containing 40 ppm lead;

Group C: nursing mothers consuming a diet containing 5 percent lead acetate, and tap water, changed at day 16 to a diet containing 40 ppm lead (*4*), and sucklings with no additional treatment, weaned on day 16 to a diet containing 40 ppm lead. Treatment of mothers with lead started on the day after birth of their offspring.

Nursing mothers and individual litters were weighed each day between 10:00 and 11:00 a.m. At 5 days of age each litter was reduced to six animals. Neonates of groups A, B, and C were allowed free access to food and water. This regimen was maintained until the end of the experiment.

Preparation of diets and analysis of brain and blood for lead were described earlier (*4, 6*). Spontaneous activity (*4*) and brain dopamine and norepinephrine (*7*) were analyzed as in the earlier report (*6*).

Body weight changes (growth) of newborn rats of the three groups are shown in Fig. 1. There was no difference in body weight gain of those young receiving daily oral doses of sodium acetate or lead acetate. They both had an average weight gain of 3 g per day from the time of birth until 34 days of age. A similar gain in daily weight has been observed in sucklings from mothers eating normal laboratory chow (*4, 5*). The newborns receiving nourishment from mothers eating a diet containing 5 percent lead acetate (group C), who are weaned to a diet containing 40 ppm lead, gain approximately 2.3 g per day, experiencing about a 30 percent depression in growth relative to groups A and B.

Treatment with sodium acetate (group A) did not have any effect on lead content of blood (0.9 μg per gram of packed cells) and brain (0.16 μg/g, wet weight) relative to that found previously in normal animals in this laboratory (*4–6*). As expected, exposure to lead results in increased concentrations of lead in blood as well as brain. Animals suckling lead-exposed mothers (group C) experienced a thirteen- and fourfold increase in the lead content of blood and brain respectively. Newborns fed daily with 1.0 mg of lead (group B) had five- and threefold increases in lead in blood and brain, respectively.