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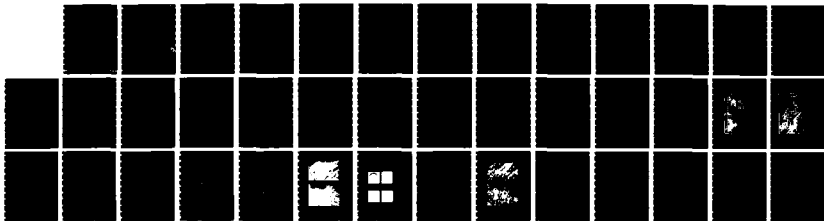
TESTS OF A MODEL FOR MACROMOLECULAR MIGRATION ON
MYOBLAST CELL SURFACES E. (U) LOMA LINDA UNIV CALIF
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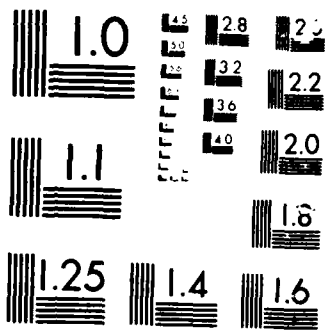
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<p>We tested the proposal (Rabinowitz, 1984) that low frequency alternating electric fields may cause a net displacement of charged cell surface molecules similar to the effect observed with static electric fields (Poo and Robinson, 1977). Spherical myoblasts obtained from cultured embryos of <i>Xenopus laevis</i> were exposed for 30 min to static fields (2.5 to 9.5 V/cm) and for 30 to 75 min to 10- and 60-Hz electric fields in the range 18 to 34 V/cm_{p-p}. Tests in electric fields were with normal medium (pH 7.8), a calcium- and magnesium-free (CMF) medium, or following pre-treatment with neuraminidase in order to investigate dependence on cell surface molecular charge. We used microfluorimetry to measure the surface distribution of concanavilin A receptors as mapped by rhodamine tagged con A. Myoblasts exposed in AC fields in normal medium</p>			
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with and without neuraminidase pre-treatment had essentially isotropic receptor distributions, contrary to expectations based on Rabinowitz's model under the assumption of certain parameter values. However, a statistical analysis indicated a small anisotropy for exposures in 10- and 60-Hz fields at 60 min ($p < .001$), although there was no evidence of receptor redistribution to the extent observable with static fields. Fluorescence micrographs revealed no clustering or clumping of receptors. Cells exposed to 60-Hz fields in CMF medium had slightly more receptor anisotropy than those in normal medium, but, if real, effects were still small. The effects in CMF appear due to a sub-population of cells for which the anisotropy was greater than under other exposure conditions, suggesting that altered divalent cation concentrations may enhance receptor motion in AC fields. Our results with static fields supported previous findings (Poo, 1981) except that "reversed asymmetry" was found only in 13% of cells treated with neuraminidase and never in normal medium. In the absence of error in the model, the insignificant receptor movement in AC fields suggests that the effective diffusion coefficient for AC fields is much less than for static fields. Perhaps the steady action of static fields more effectively disrupts molecular anchorages to the cytoskeleton.

To further investigate the possibility that the absence of significant receptor motion is attributable to a low density of charge on myoblast cell surfaces, we determined the density of cell surface binding sites for cations using cationized ferritin. Our value, 6.5 ug Fe/mg protein, was 11% of that found in rat synaptosomes, but upon correction for the larger myoblast diameter, the amount per unit area of membrane is consistent.

TESTS OF A MODEL FOR MACROMOLECULAR MIGRATION ON MYOBLAST
CELL SURFACES EXPOSED TO ALTERNATING ELECTRIC FIELDS

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Authors: S. Lin-Liu, Ph.D. and A.R. Sheppard, Ph.D.

INTRODUCTION: The response of cell surface charged macromolecules to static (DC) electric fields has been successfully explained by the model of "in situ electrophoresis" (Poo, 1981). While cells may experience static electric fields during embryogenesis (Jaffe and Stem, 1979) or in a laboratory setting, fields encountered in vivo more often have pulsed or sinusoidal waveforms. Recently, Rabinowitz (1984) proposed a model which considers the motion of cell surface charged macromolecules under the influence of low frequency AC electric fields. The macromolecules are located on an immobile hemispherical surface which may be taken to represent the hemispherical surface of a cultured cell attached to a substrate. The model predicts that surface charged molecules redistribute in an AC electric field with a resulting accumulation of molecules on the parts of the surface perpendicular to the field and with a corresponding loss of molecules from regions parallel to the field. As viewed from a point above the substrate, molecules would accumulate along the cell periphery at poles defined by the field axis. Viewed parallel to the plane of the substrate, molecules would concentrate in the region where the cell contacts the substrate. The degree of predicted concentration gradient depends on the charge density and diffusion coefficient of the affected molecules.

The topographic change in charged molecules on a cell surface may have a significant functional consequence, for example, as in the accumulation of acetylcholine receptors at neural and neuromuscular junctions (Elson and Schlessinger, 1979). In the antigenically stimulated mast cells of the immune system, release of histamine was found to follow the lateral movement of the antigen-antibody complex (Siegel et al., 1976). We began these studies with the expectation that improved understanding of the field responses of surface macromolecules may also improve understanding of the effects of low intensity, extremely low frequency electromagnetic fields reported in several in vitro biological systems (Bawin and Adey, 1976, Bawin et al., 1978, Blackman et al., 1982, Lin-Liu and Adey, 1982, Luben et al., 1982, Lyman grover et al., 1983, Lyle et al., 1983, Raybourn, 1983). These reports suggested the prime site of field-tissue interaction is at the cell surface.

In this report we examine the cell surface dynamics in AC electric fields using single cell cultures of myoblasts from Xenopus laevis. This preparation has been previously used to study effects of DC (Poo and Robinson, 1977, Orida and Poo, 1978) and pulsed (Lin-Liu et al., 1984) electric fields. These studies demonstrated that this system is suitable for AC field studies and provided the following significant information:

1) Concanavalin A (con A) receptor molecules respond to both DC and pulsed electric fields, an indication of the charged nature of the receptors; 2) Prefield molecular distribution over the cell surface is uniform, thereby allowing observation of possible field-induced anisotropy; 3) After field exposure, the receptor molecules are free to move in the plane of the membrane with a diffusion coefficient similar to that found for other surface molecules; 4) Cells attach to the substrate firmly and do not migrate or reorient in DC electric fields. These geometrical and physical properties appear to meet the requirements of Rabinowitz's model (see also Rabinowitz, 1982) and thus emphasize the suitability of the myoblast model for this study. Furthermore, the hemispherical cell geometry permits experimental and theoretical quantification of molecular concentration on the cell surface.

In the present study we used established techniques of microfluorimetry and fluorescence microscopy to examine for sensitivity of con A receptor redistribution on the myoblast cell surfaces exposed to AC fields. Since the extent of the predicted AC field effect depends on the magnitude of the molecular charge density and molecular mobility (Rabinowitz, 1984) we manipulated these factors with neuraminidase and by using a Ca^{2+} - Mg^{2+} -free medium. We also conducted biochemical assays with cationized ferritin to measure surface charge in normal medium.

In summary, the present study had two goals: 1) to assess possible AC field-induced anisotropy in con-A receptor distribution; and 2) to evaluate the significance of surface charge in the influence of AC electric fields.

MATERIALS AND METHODS:

A) Fluorescence Microscopy of Electric Field-Exposed Cultured Myoblasts.

Myoblast single cell cultures on cover slips were made from *Xenopus laevis* (Nasco Corp., Fort Atkinson, WI) embryos at stages 17-19 (Fig. 1). Cultures were used at age 35-45 h. Culture techniques and electrophoretic procedures were similar to those described previously by Poo, et al. (1979). Briefly, electrophoresis chambers (Fig. 2) were made from glass slides and cover slips. Electric current was applied at room temperature to the electrophoretic chamber either through agar bridges (DC) or sheet platinum electrodes (AC). Experiments were performed in a Faraday cage. Controls were treated identically but without current flow. Exposure conditions are further detailed in the Results and Discussion section below. Following field exposure, cultures were immediately labeled with con A conjugated with tetramethylrhodamine isothiocyanate (R-con A) (Vector Lab Inc., Burlingame, CA). R-con A labeling was carried out at 4°C for 10 min in modified Steinberg's saline (Jones and Elsdale, 1963), buffered with 10 mM HEPES (Sigma Chemical Co., St. Louis, MO) and containing 15 ug/ml R-con A and 0.1% bovine serum albumin (BSA) (Sigma).

Cells were then rinsed thoroughly at 4°C with Steinberg-BSA followed by Steinberg's saline alone, and examined with a fluorescence microscope.

Cell surface charge was manipulated by either of these procedures: a) Cells were pre-treated for 30 min with neuraminidase by incubation in Steinberg's saline containing 5 units/ml of Vibrio cholerae neuraminidase (Calbiochem, San Diego, CA) at pH 6.8 at room temperature. They were then washed thoroughly with Steinberg's saline and immediately exposed to field or control (i.e., no field) conditions. Following labeling with R-con A, both field-exposed and unexposed cells were examined for intracellular fluorescence which would indicate damage to the cell membrane, but none was found. Or, b) cells were pre-treated with a Ca²⁺- Mg²⁺-free (CMF) Steinberg's saline containing 1 mM EGTA beginning 10 min before field application and continuing throughout the exposure. Cells were then labeled with R-con A as described above.

Data collection was carried out by microfluorimetry and fluorescence photography.

1) Microfluorimetry. Following con A receptor labeling, fluorescence intensity over the cell periphery was viewed with a Zeiss inverted fluorescence microscope (Model IM 35, Carl Zeiss, Inc., Thornwood, NY) fitted with a Zeiss PM1 photometer. Fluorescence intensity was measured using a 9.0 um diameter aperture at four positions on the cell periphery. These points lie along membrane surfaces perpendicular or parallel to the field direction. With the field directed along the 0-180 degree axis (x-axis), the four measured sites had the angular positions of 0, 90, 180 and 270 degrees (Fig.3). Background intensity for a cell was taken at an arbitrary point about two cell diameters from the cell surface. Three distribution indexes, namely,

$$A_x = (I_0 - I_{180}) / (I_0 + I_{180}),$$

$$A_y = (I_{90} - I_{270}) / (I_{90} + I_{270}),$$

$$A_{xy} = (I_0 + I_{180} - I_{90} - I_{270}) / (I_0 + I_{180} + I_{90} + I_{270}),$$

were calculated to express the anisotropy of the con A receptor distribution at the cell surface. A_x , A_y and A_{xy} respectively represent the asymmetries in the directions parallel or perpendicular to the field and the differential asymmetry in the two directions. I_0 , I_{90} , I_{180} and I_{270} are the corrected (background subtracted) fluorescence intensities at the four positions. Non-zero values for any index indicate anisotropic receptor distributions at the cell surface.

2) Fluorescence Photography. Fluorescence images of R-con A bound to cells were recorded on Kodak Tri-X Pan film pushed to ISO 800. The photographs were examined for possible microscopic fluorescence clusters or molecular aggregates.

B) Cell Surface Charge.

Myoblast surface charge was assessed by an assay for cationized-feritin (CF) binding (Lin-Liu and Bondareff, 1981) to Xenopus embryonic cells. However, single cells were kept in suspension instead of being

plated on glass, in contrast to studies with electric fields. Prior to incubation in CF, dissociated cells were allowed to remain in culture medium for 2 h at room temperature to recover from the trauma of dissection. Since we were interested in detecting CF binding to the cell surface, all CF labeling procedures were carried out at 4°C to prevent endocytosis of CF at higher temperature. The cell suspension was centrifuged at 2000 g for 10 min and the pellet was suspended in Steinberg's saline at a concentration of about 0.5 mg protein/ml. Aliquots of the suspension were then added to an equal volume of CF solution and incubated (at CF concentrations of between 0.25 and 2.0 mg/ml in Steinberg's solution) for durations of 1 to 30 min. The level of nonspecific binding was obtained using corresponding concentrations of natural ferritin without incubation. The mixture was then diluted 5 fold with Steinberg's saline and centrifuged at 2000 g for 10 min. The pellet was thoroughly rinsed with Steinberg's saline followed by the addition of a small volume of concentrated formic acid. The amount of iron in the test tubes was determined (see below) as a measure of bound CF. The level of binding was expressed as micrograms of iron per milligram tissue protein ($\mu\text{g Fe/mg}$).

The CF binding assay was also used to determine the effects of neuraminidase on cell surface charge. Prior to the initial centrifugation at 2000 g, cells were treated with neuraminidase (prepared as above) for 30 or 60 min.

C) Iron and Protein Determinations.

Full details are found in previously published work (Lin-Liu and Bondareff, 1981). Briefly, oxidation of iron in the presence of diprydil and NaSO_3 in acidic solution turned the solution red. Colorimetric reading of the solution at a wavelength of 520 nm gave a linear response in proportion to iron concentrations between 1 and 17 $\mu\text{g/ml}$.

Protein amounts were determined by Bradford's method (1976).

D) Electron microscopy.

Binding sites of CF were examined by transmission electron microscopy. Following CF binding, cells were fixed in 2% glutaraldehyde and 1% sucrose in Steinberg's saline, postfixed in 1% OsO_4 buffered with sodium cacodylate, dehydrated in a graded series of alcohols, and embedded in Epon in the conventional way. Unstained thin sections of 700 nm were examined with a Zeiss electron microscope.

E) Field Exposure Conditions.

1. DC Field. Cultured single cells were exposed for 30 min to DC electric fields of intensity 2.5-9.5 V/cm followed by R-con A labeling and microfluorimetry.

2. AC Field. Sinusoidal symmetric AC electric currents at 10- and 60-Hz were used. Cells were exposed at room temperature for 60 min in most cases. Exposures of 30, 45 and 75 min were also used for comparison. Electric field gradients fell into two ranges, 18-24 and 28-34 V/cm_{p-p}.

F) Statistical Tests.

Statistical tests were made using the BMDP Statistical Software (Dixon et al., 1981) programs P1D, P3D, P4D, P5D, P4F and P2V for general statistical measures, analyses of variance (ANOVA), Student's t-tests, and comparisons of distributions.

RESULTS AND DISCUSSION:

DC Field Electrophoresis. The results in DC fields provide information on the diffusion coefficient and serve as a positive control to show the response of con A receptors to DC electric fields. Cells were exposed in Steinberg's saline. Receptor distribution was polarized toward the cathode. Fig. 4 shows the distribution index along the direction of the electric field (A_x). Electrophoretic response of con A receptors was easily detected at 2.5 V/cm with a typical index A_x of 0.13. A plateau was approached at field intensity of about 5 V/cm and the maximal distribution index was 0.37, seen at 9.5 V/cm. These results were essentially the same as those previously published by Poo et al. (1979, 1981) and indicated a diffusion coefficient of similar magnitude ($\approx 5 \times 10^{-9}$ cm²/s).

AC Field Exposure and Con A Receptor Distribution. Con A receptors were labeled immediately following exposure in Steinberg's saline. From each slide distribution indices were obtained for about 20-30 cells, and these were combined to form a mean and standard deviation (SD) for each slide. A total of 11 slides (253 cells) was measured for the control condition, 14 slides (315 cells) for cells exposed at 10-Hz, and 10 slides (227 cells) exposed at 60-Hz. The majority of the data were obtained following 30 and 60 min exposures, but a few exposures lasted 45 and 75 min. Tables 1 to 3 list the results. In each control slide, all three distribution indexes were within two standard deviations of zero. The same can be stated for the exposed samples at either of the frequencies and either of the field strength ranges used. Thus all three distribution indexes indicated an isotropic receptor distribution for control and AC field-exposed cells. These results are plotted in Fig. 5 to demonstrate the similarity of control and exposed slides.

Additional statistical tests were made with the data collected from 30 and 60 min exposures. Table 5 summarizes the results in which data were averaged taking the cell, rather than the slide, as the experimental unit. There was complete overlap of all categories with the exception of the control data obtained after 60 min sham exposures. Analysis of variance (Table 6) showed significant interactions for the effects of time (30 vs 60 min) and solution (normal and CMF media) as well as second order effects for field with time and field with solution. The field itself was

not a significant factor. The source of these interactions is evident in Table 7 which shows t-tests between the various categories. Significant effects occurred wherever another condition was compared to the 60 min control group. In addition, the t-test for the 60-Hz, 30 min, CMF condition was significant ($p < .04$). It should be noted that most data were obtained with 60 min exposures and that the standard deviation in the data was consistently about 0.04, suggesting good experimental technique. The fact that values of A_x and A_{xy} were consistently distributed slightly above zero may be due to systematic operator errors, but systematic equipment error is unlikely because microscope alignment was carefully checked daily.

We suspect that the indications of statistically significant effects are spurious. This interpretation is enforced by: 1) the small size of any possible differences compared to effects observed with static fields and compared to the standard deviation in the data; 2) the occurrence of a "significant" effects in the control data which may merely reflect the vagaries of cell culture conditions, fluorescence microphotometry and non-equivalent experiments done at different times; 3) the non-conservative choice of values of "n" from tallies of all cells in a group. Nonetheless, these results may point to some small effects which cannot be reliably detected with the experimental techniques of this study.

In summary, under the conditions used in this experiment, con A receptors did not show the AC field-induced movement predicted by Rabinowitz's model assuming particular values for several parameters. Although we obtained statistically significant effects for tests with 10- and 60-Hz fields acting for 60 min, but not for 30 min, this is not evidence of significant receptor motion.

Important parameters in Rabinowitz's model are cell dimension, field intensity, diffusion coefficient of the molecules, and the anisotropy is especially sensitive to the magnitude of the charge carried by the molecules. Frequency of the AC field was not significant and exposure duration longer than 30 min was considered sufficient. In our experiment, no effect of cell size was observed with average cell diameters on each slide ranging from 32.3 to 40.4 μm (Tables 2, 3). Field intensities used here were within the range for which the model predicts significant molecular movement assuming the myoblast diffusion coefficient found by Poo ($\approx 5 \times 10^{-9} \text{ cm}^2/\text{s}$) and a molecular charge per molecule of about five electron units (Rabinowitz, 1984). Our failure to detect an AC field effect here occurred despite evidence which confirms the DC electrophoretic behavior of con A receptors. However, an estimate of the charge per molecule from the DC studies indicates only about 2 electron units per molecule. We also did additional experiments to ascertain 1) if, in contradiction to our assumptions, surface charge density was unexpectedly low; or 2) if local inhomogeneities occurred without net receptor movement. The chemical and microscopic tests for these factors are described below.

CF-Binding to Myoblast Cell Surfaces. CF bound to myoblasts in a time- (Fig.6) and dose-dependent (Fig. 7) fashion, reaching a plateau after a 20 min incubation. Dose response at 20 min incubation saturated at CF concentrations slightly above 1 mg/ml. Maximal binding capacity was about 6.5 ug Fe/mg protein. Electron microscopic examination of the cells following CF binding confirmed that CF did not penetrate the cell membrane and it bound solely to the cell surface (Fig. 8). Therefore, the amount of charge on the cell surface is reflected by the amount of bound CF as determined by the amount of iron present.

Although measurements of the absolute amount of surface charge are not possible from these results, the relative magnitude of the charge density can be obtained by comparison with results from synaptosomal membrane. Previous experiments showed that the maximal binding capacity of synaptosomes was about 60 ug Fe/mg protein (Lin-Liu and Bondareff, 1981), 9 times higher than was found here. However, comparison of surface density should be made with the CF bound per unit surface area. For discussion purposes, we assume cell size to be uniform and refer to both myoblasts and synaptosomes as cells. The surface area of a cell is proportional to the square of the cell diameter. By assuming identical tissue densities in myoblasts and synaptosomes, the number of cells per unit volume is inversely proportional to the cube of cell diameter and the total surface area per milligram of tissue is inversely proportional to cell diameter. Therefore, the amount of CF bound per unit surface area is proportional to the product of CF bound per milligram of tissue and cell diameter. The diameter of spherical myoblast single cells in suspension is typically 7-10 um and of synaptosomes 0.6-0.9 um, yielding a ratio of diameters consistent with the aforementioned quotient of 9, and leading to the result that the CF bound per unit surface area is comparable in the two preparations. This suggests that membrane surface charge densities are comparable for myoblasts and synaptosomes. Since neuronal membrane surface is believed to have a surface charge typical of other cells (Elul, 1967), the myoblast cell membrane charge density appears to be typical of all cells. However, our failure to observe significant receptor motion may be due to a low surface charge carried by the con A receptors when compared to the value of 5 electronic charges per molecule assumed in calculations based on Rabinowitz's model.

Neuraminidase treatment for 30 min prior to CF binding reduced the amount of bound CF in cell suspension from 4.46 ± 0.04 (SD) to 3.21 ± 0.01 ug Fe/mg, a 28% reduction. Prolonged treatment up to one hour only led to a small additional reduction (9%). Since neuraminidase removed sialic acids from the surface of cells during incubation, we assume that similar effects occurred in the plated cells. Therefore incubation for 30 min was used in further experiments.

Field Exposure Following Neuraminidase Treatment. Both DC and AC fields were used in this study. Following DC field exposure at 5 V/cm for 30 min, the con A receptor distribution became polarized. The extent of overall asymmetry (A_x) was 0.191 ± 0.140 (mean \pm SD) in 56 cells. The mean value was slightly reduced (32%) in comparison with controls which received no neuraminidase treatment (Fig. 4).

However, as indicated by the large standard deviation, we saw a striking effect: reversed motion of the receptors. Although the con A receptor concentration remained highest on the surface facing the cathode for all 234 cells of Fig. 4, in 7 of 56 neuraminidase-treated cells (13%) the asymmetry was reversed (receptor accumulation toward the anode). These results are not in total agreement with those reported by Poo (1981), who found reverse asymmetry of -0.28 ± 0.05 (mean \pm SEM) in 54 neuraminidase-treated cells exposed to 10 V/cm for 30 min compared to 0.41 ± 0.03 in 122 cells in normal medium. This discrepancy between Poo's study and our own cannot be explained by the difference in field intensity used because the direction of molecular migration depends only on molecular charge and the direction of the field. Differences in cell surface properties may be responsible for the discrepancy. We observed no reverse asymmetry in the normal medium, but Poo infrequently found such an effect (personal communication). Although enzyme action was nearly complete within the incubation period, we used a different source (Calbiochem) compared with Poo (Sigma). We conclude that the reversed response to electric fields indicates that our myoblast cultures contain a subpopulation with opposite surface dynamics which becomes apparent after neuraminidase treatment.

AC electric currents at 60-Hz were applied to neuraminidase-treated cells. Table 4 presents the distribution indices from these experiments. For each slide, all three distribution indices were within one standard deviations of zero, as previously found in the no field condition (Table 1). Therefore, our data indicate that removal of sialic acids from cell surfaces did not enhance sensitivity to AC fields.

Field Exposure in Ca^{2+} - Mg^{2+} -Free Media. DC fields induced receptor accumulation toward the cathode, as observed in control experiments in regular Steinberg's saline. However, the degree of asymmetry was greater. Exposures at 2 and 3 V/cm produced asymmetry indexes, A_x , of 0.23 ± 0.09 (mean \pm SD, 26 cells) and 0.30 ± 0.09 (21 cells), respectively. These values fall well above the curved line for the controls in Fig. 4. Thus, removal of divalent cations from the medium increased the responsiveness of con A receptors to DC fields by about 100% at these intensities. At a higher field intensity, 10 V/cm, Poo (1981) found similar effects, but to a lesser extent (20%).

AC field exposure did not give consistent results for A_{xy} , the parameter which represents con A receptor anisotropy over the cell surface. In one experiment at 60-Hz, the mean value was 0.085, more than two standard deviations above zero (Table 4). However, in other experiments the results suggested little or no asymmetry. The anova and t-test results indicated effects for both 10- and 60-Hz fields, but these findings are subject to the same cautions expressed for findings in normal media. We conclude that removal of divalent cations did not significantly enhance AC field-sensitivity. However, the distribution of A_{xy} (Fig. 10) suggested there were two populations, one responsive to the AC field and another unresponsive. Exposures for 30 min (60-Hz) in CMF medium had a single mode centered on the relatively high mean value of 0.047.

Fluorescence Photography. Photographs of the fluorescence images of R-con A binding cells were examined in some experiments. The plane of focus was held at the periphery of the cells for analysis of possible receptor clustering or other forms of anisotropic distribution at the cell surface. A total of 71 control cells, 70 cells exposed to 10-Hz, and 140 exposed to 60-Hz was examined. Consistently uniform receptor distribution was found in both controls and AC field exposed cells. Further, no differences in cell appearance or uniformity of fluorescent staining were detected between the controls and field exposed cells (Fig. 9). Therefore, at the light microscope level, AC fields did not induce formation of local molecular aggregates.

SUMMARY:

Under the conditions of the present experiment, con A receptors did not show AC electric field-induced redistribution on the cell surface as predicted by Rabinowitz's model. Very slight differences in receptor distribution were found by statistical tests of 60 min exposures to 10- and 60-Hz and similarly small effects were also determined in field tests with Ca^{2+} - Mg^{2+} -free solutions. Higher field intensity and longer exposure duration were avoided to minimized possible immobile receptor aggregate formation (Poo, 1980).

The molecular system used was previously shown to have a rather high diffusion coefficient that should have facilitated the action of AC electric fields. The possibility exists that the diffusion coefficient for large displacements obtained in DC electrophoresis studies is not applicable to the AC field situation. The charge of con A receptors is the most important parameter which can significantly alter the quantitative predictions of Rabinowitz's model. Although precise data of cell surface molecular charge density are very difficult to obtain, our measurements of CF binding are consistent with established values. Thus, in the absence of error in the model, the lack of significant receptor movement in AC fields suggests that the effective diffusion coefficient for AC fields is much less than for static fields. One consideration which is outside the scope of the model or this study is that perhaps the steady action of static fields is more effective in disrupting the molecular anchorages to the cytoskeleton in comparison with the oscillatory forces developed by extremely low frequency AC fields.

Our results with static fields supported previous findings (Poo, 1981) except that "reversed asymmetry" was found only in 13% of cells treated with neuraminidase and never in normal medium, in contrast to previous data.

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Legends

Table 1. Microfluorimetry measurements of R-con A receptor distributions. The three distribution indexes A_x , A_y and A_{xy} (mean \pm SD) obtained from cells exposed to control conditions (0 V/cm) for the indicated number of minutes (t). The number of cells per slide and average cell diameter ($d \pm$ SD) in micrometers are also given.

Table 2. As in Table 1 for exposures to a sinusoidal symmetric 10-Hz E-field at the field conditions shown.

Table 3. As in Table 1 for exposures to a 60-Hz E-field at the field conditions shown.

Table 4. Distribution indexes (mean \pm SD) for field-exposed myoblasts. Neuraminidase-pretreated cells (5 unit/ml, 30 min, pH 6.8) were exposed in regular Steinberg's saline (pH 7.8). Ca^{2+} - Mg^{2+} -free (CMF) medium (pH 7.8) was applied 10 min prior to and during exposure.

Table 5. Summary of average values of A_{xy} . Standard deviations (SD) and number of tested cells (n) are shown for each experimental condition. Data derived from Tables 1-3.

Table 6. Results of two-way ANOVA among all conditions. Note, in the absence of separate control data in CMF medium, controls in normal solution were used.

Table 7. Student's t-tests among groups of test conditions. NS indicates $p > .05$. The cell numbers, n and m apply to the paired groups respectively. Significant effects were observed in all contrasts with the 60 min control data. Other tests (not shown) included insignificant differences between 10- and 60-Hz conditions.

Figure 1. A pair of Xenopus laevis is shown in the trough in which they are kept. Fertilized eggs are collected under the grid of size 1 x 1 cm.

Figure 2. Electrophoretic chambers were made from 7.6 x 2.5 cm microscope slides shown at the center of the picture. Alternating current was applied through the pair of sheet platinum electrodes. For studies with direct current, agar bridge electrodes were interposed between the chamber and the platinum.

Figure 3. Schematic of a myoblast cell exposed to an electric field (E). The cell diameter is approximately 35 μ m and fluorescence intensity was measured within a 9 μ m aperture shown at four locations on the cell surface. Membranes at 90 and 270° were parallel to the E-field and those at 0 and 180° were perpendicular. For DC fields the zero degree position faces the cathode.

Figure 4. Variation of receptor asymmetry as measured by the distribution index A_x is shown for cells exposed to a static electric field for 30 min. The numbers in parenthesis give the numbers of cells at each field strength, error bars show SD, and the dashed line indicates the 0 V/cm level.

Figure 5. Distribution indexes listed in Table 1, 2 and 3 are plotted. A. The indexes A_x , A_y and A_{xy} (see text) obtained from a number of cells (about 20-30) on each of a number of slides are shown for data obtained under control conditions (no electric field). B. Distribution indexes obtained from cells exposed at 10-Hz over the range of field strengths cited in the text. Although slightly skewed above zero, by comparison with effects of a DC field the data show no significant asymmetry in receptor distribution. C. Similar results were found for the 60-Hz exposures. Notice the similarity in A_x , A_y and A_{xy} among the three conditions.

Figure 6. Time Course of Cationized Ferritin (CF) Binding. Suspended myoblasts were incubated in Steinberg's saline containing 0.5 mg/ml CF at pH 7.8 for 2.5, 5, 10, 15, 20 and 30 min. Binding reached a plateau after about 20 min.

Figure 7. Dose Dependence of CF Binding. Suspended myoblasts were incubated for 20 min at pH 7.8 in Steinberg's saline containing various concentrations of CF. Binding levels reached a plateau value of about 6 μ g iron per mg protein for CF concentrations greater than 1 mg/ml.

Figure 8. Electron Micrographs of Portions of Two Cultured Myoblasts. Sectioned specimens were not counter-stained in uranyl acetate and lead citrate. Cells incubated for 20 min in pH 7.8 Steinberg's saline without CF (A) and with 0.5 mg/ml CF (B). CF was bound only at the cell surface (open arrow) and not in intracellular membrane structures such as the mitochondria shown here (closed arrow). Thin arrows indicate ribosomes. Uncalibrated magnification.

Figure 9. Fluorescent images of *Xenopus* myoblasts. A) Control. B) Following 30 min exposure to a static electric field (9.5 V/cm) along the direction shown. Receptors moved toward the cathodal pole. C) Following 60 min exposure to a 10-Hz electric field (34 V_{p-p} /cm), no receptor redistribution was observed. D) As in C, following a 60-Hz exposure for 60 min (28 V/cm_{p-p}). Calibration bar in A shows 25 μ m.

Figure 10. Distribution of A_{xy} for cells exposed to electric fields in Ca^{2+} - Mg^{2+} -free medium and in medium containing neuraminidase. The data for both A (CMF medium, 10-Hz field) and B (neuraminidase, 60-Hz) suggest two sub-populations, one responsive to the field and the other unresponsive. For comparison, C shows cells exposed to 60-Hz in normal medium and D shows the distribution for unexposed cells in normal medium.

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TABLE 1

Slide #	E (V/cm)	t(m)	A _x	A _y	A _{xy}	d(um)	n
1	0	60	.066 ± .051	-.004 ± .061	.039 ± .029	41.3 ± 1.9	21
2		60	.006 ± .016	-.007 ± .160	.005 ± .015	38.8 ± 2.0	24
3		60	-.010 ± .112	-.017 ± .059	.009 ± .075	--	40
4		60	.051 ± .054	.000 ± .064	.026 ± .036	--	30
5		60	.018 ± .057	-.006 ± .060	.012 ± .031	--	18
6		60	.027 ± .077	-.018 ± .076	.007 ± .032	33.7 ± 3.0	15
7		60	-.005 ± .016	.018 ± .030	.005 ± .020	32.7 ± 2.7	18
8		60	.017 ± .026	-.006 ± .032	-.006 ± .033	32.6 ± 2.2	15
9		60	.057 ± .068	.006 ± .045	.035 ± .048	33.0 ± 2.9	24
10	0	30	-.026 ± .051	.005 ± .058	.033 ± .039	--	23
11		30	-.002 ± .044	.005 ± .056	.048 ± .025	--	25

TABLE 2

Slide #	E (V/cm)	t (m)	A _x	A _y	A _{xy}	d (um)	n
1	18-24	30	.073 ± .040	.006 ± .027	.046 ± .032	39.2 ± 3.1	28
2		30	.019 ± .040	.005 ± .048	.018 ± .035	36.6 ± 2.5	26
3		45	-.008 ± .020	.041 ± .025	.005 ± .011	37.1 ± 2.8	27
4		60	.057 ± .033	-.002 ± .028	.047 ± .027	40.3 ± 1.9	32
5		60	.022 ± .058	-.001 ± .044	.024 ± .042	33.9 ± 1.3	22
6		60	-.010 ± .010	.015 ± .025	.005 ± .011	34.2 ± 3.0	15
7		60	.025 ± .037	-.004 ± .027	.014 ± .041	32.2 ± 2.1	22
8	28-34	45	-.001 ± .021	.029 ± .027	.005 ± .013	32.5 ± 2.4	13
9		60	.017 ± .049	.000 ± .051	.050 ± .053	33.7 ± 2.3	24
10		60	.021 ± .031	-.002 ± .028	.017 ± .030	33.5 ± 2.1	25
11		60	-.004 ± .077	-.009 ± .061	.025 ± .045	32.7 ± 2.1	20
12		60	.011 ± .068	.004 ± .057	.046 ± .060	32.9 ± 2.0	20
13		75	-.007 ± .015	.025 ± .017	.005 ± .010	34.1 ± 3.0	16
14		75	-.011 ± .023	.022 ± .020	.000 ± .017	33.7 ± 1.8	24

TABLE 3

Slide #	E (V/cm)	t (m)	A _x	A _y	A _{xy}	d (um)	n
1	18-24	30	.063 ± .034	.007 ± .024	.034 ± .024	40.4 ± 4.1	28
2		30	-.039 ± .059	.065 ± .079	.028 ± .027	--	15
3		60	.041 ± .023	.008 ± .023	.025 ± .017	38.9 ± 3.2	25
4		60	-.012 ± .024	.024 ± .025	.008 ± .008	33.9 ± 1.9	14
5		60	.034 ± .066	-.009 ± .060	.058 ± .044	34.7 ± 2.9	24
6		75	-.025 ± .074	.012 ± .035	.006 ± .032	33.6 ± 2.1	14
7	28-34	60	.025 ± .041	-.018 ± .043	.022 ± .040	32.7 ± 1.8	32
8		60	.048 ± .063	-.011 ± .040	.064 ± .046	33.8 ± 2.2	29
9		60	.020 ± .022	-.003 ± .028	.036 ± .030	34.3 ± 1.9	19
10		60	.078 ± .065	-.002 ± .031	.042 ± .037	32.3 ± 2.2	27

TABLE 4

Treatment	Freq (Hz)	Time (min)	A _x	A _y	A _{xy}	n
Neuraminidase	60	30	-.014 ± .050	.031 ± .105	.017 ± .046	17
			-.019 ± .037	.013 ± .085	.040 ± .048	19
			.025 ± .086	.005 ± .066	.023 ± .049	27
			-.007 ± .040	.020 ± .058	.027 ± .035	26
Ca ²⁺ -Mg ²⁺ -free	10	30	.034 ± .058	-.015 ± .057	.030 ± .052	23
		60	.066 ± .053	-.039 ± .081	.034 ± .044	18
	60	30	.027 ± .033	-.001 ± .034	.010 ± .027	18
		60	.052 ± .075	-.024 ± .054	.064 ± .062	25
	60	30	.030 ± .052	.026 ± .056	.085 ± .034	18
			.019 ± .063	.036 ± .048	.044 ± .042	22
			.019 ± .059	-.001 ± .060	.035 ± .029	25
			-.011 ± .048	.014 ± .065	.029 ± .045	19

Note: Field amplitude = 28 to 34 V/cm-p.

TABLE 5

Conditions	A _{xy} ± SD	n
Control, 30 min/Normal Sol.	.041 ± .033	48
Control, 60 min/Normal Sol.	.016 ± .046	205
10-Hz, 30 min/Normal Sol.	.033 ± .036	54
10-Hz, 60 min/Normal Sol.	.031 ± .043	181
60-Hz, 30 min/Normal Sol.	.034 ± .032	43
60-Hz, 60 min/Normal Sol.	.038 ± .039	176
10-Hz, 30 min/CMF Sol.	.030 ± .052	23
10-Hz, 60 min/CMF Sol.	.039 ± .053	61
60-Hz, 30 min/CMF Sol.	.047 ± .042	84
60-Hz, 30 min/NA Sol.	.026 ± .044	89

TABLE 6

Interaction Tested	Significance
Field	NS
Time	p < .05
Solution	p < .05
Field x Time	p < .01
Field x Solution	p < .002
Solution x Time	N.S.
Field x Time x Solution	NS

Note: Fields are Control, 10- and 60-Hz; times are 30 and 60 min; solutions are Normal and CMF.

TABLE 7

Groups Contrasted	t	n, m	p
10-Hz, 30 min vs Control, 30 min	1.18	54, 48	N.S.
10-Hz, 60 min vs Control, 60 min	-3.37	181, 205	< .001
60-Hz, 30 min vs Control, 30 min	.97	43, 48	N.S.
60-Hz, 60 min vs Control, 60 min	-5.06	176, 205	< .001
CMF vs Normal Control Solution:			
10-Hz, 30 min vs Control, 30 min	.86	22, 48	N.S.
10-Hz, 60 min vs Control, 60 min	-3.13	61, 205	< .002
60-Hz, 30 min vs Control, 30 min	-0.86	84, 48	< .04
60-Hz, 60 min vs Control, 60 min	--	--	--



Figure 1

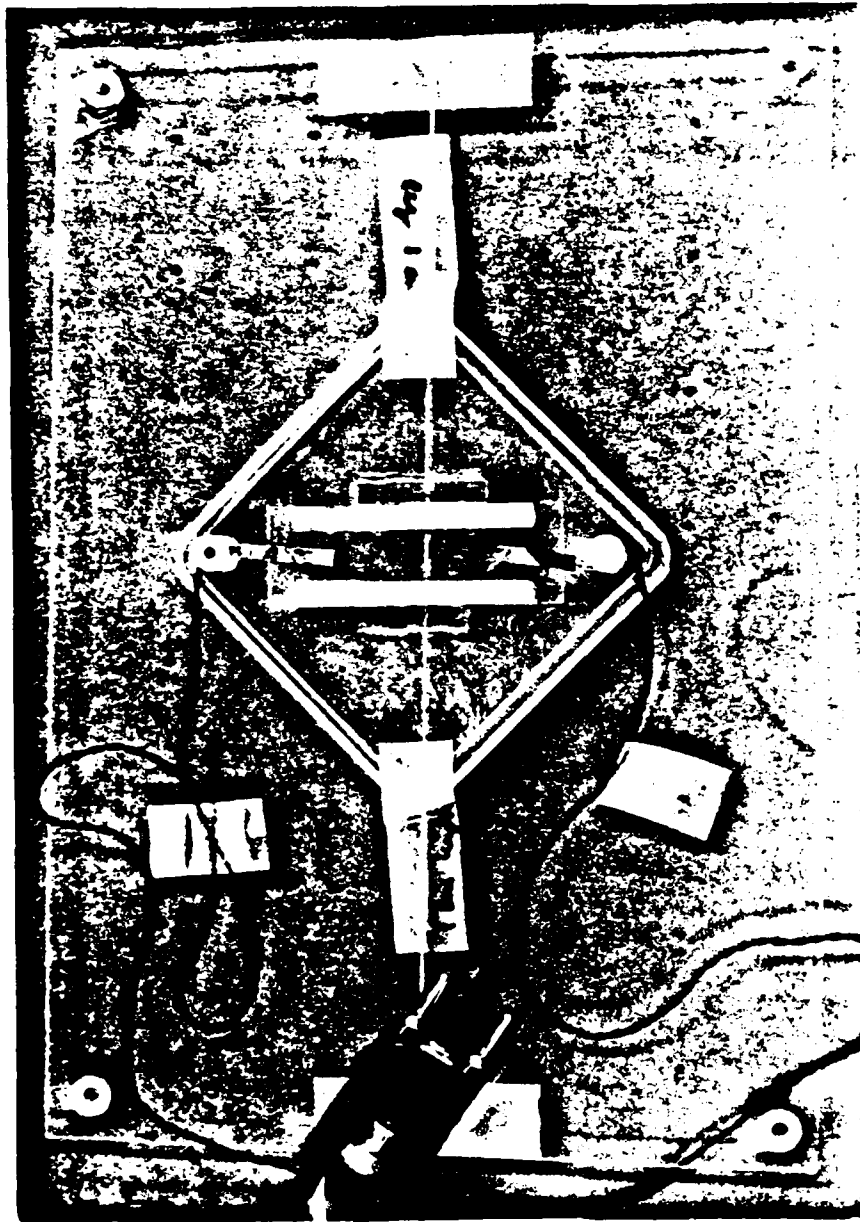


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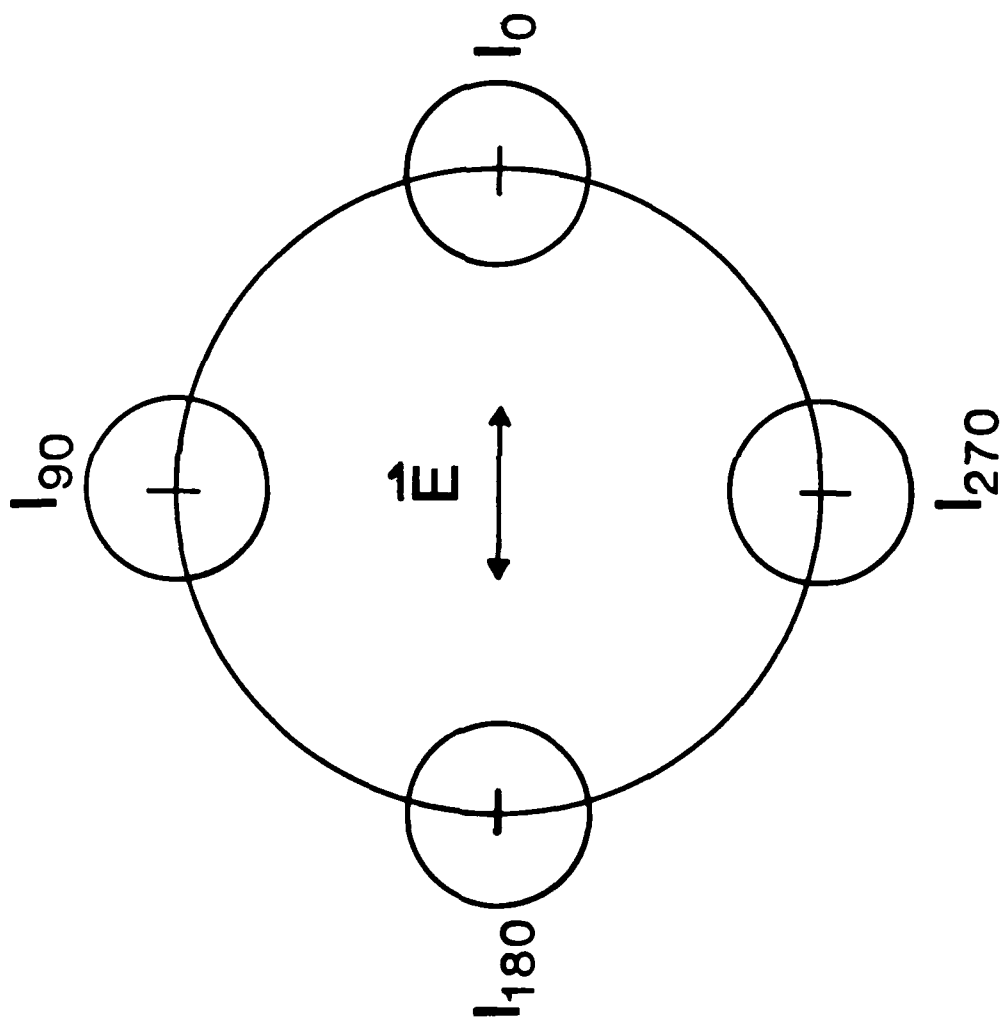


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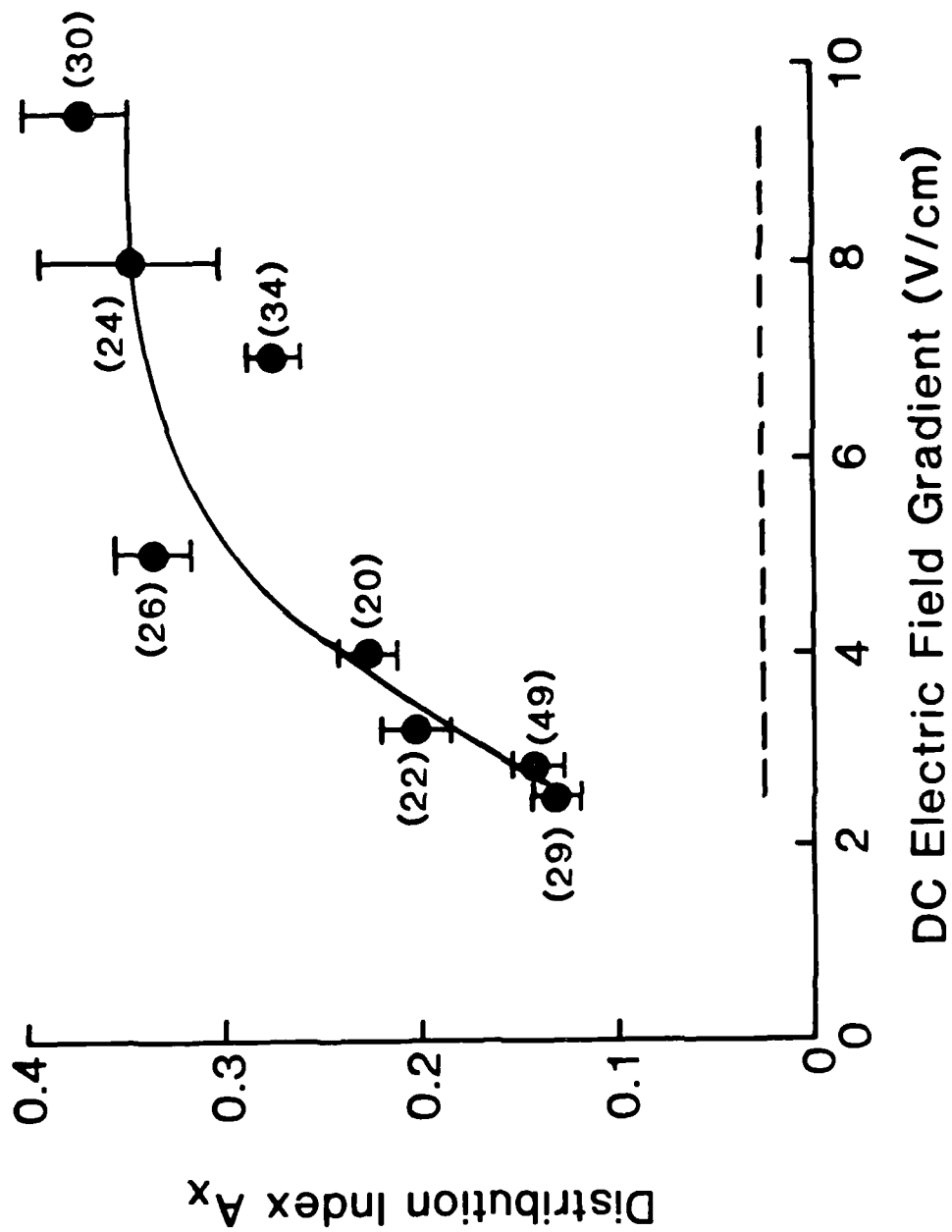
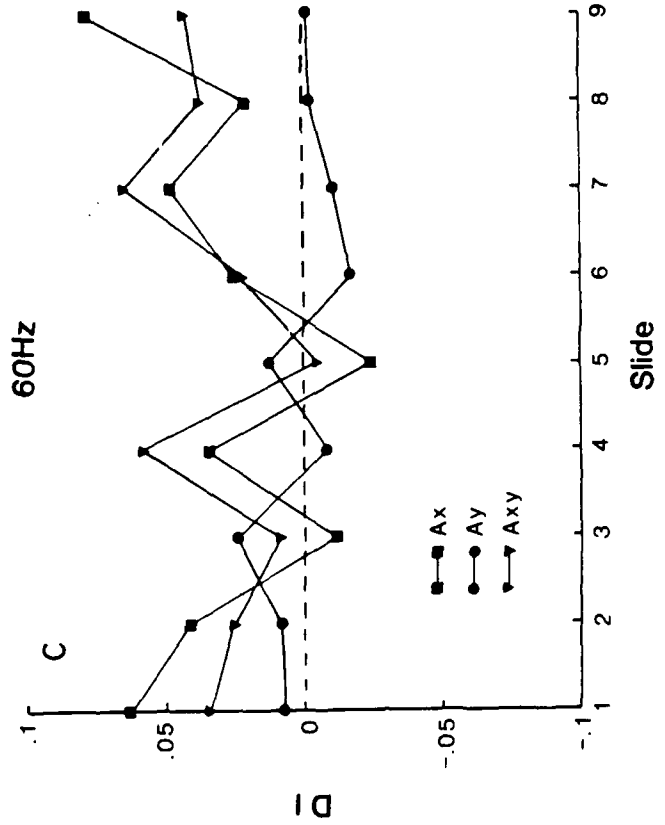
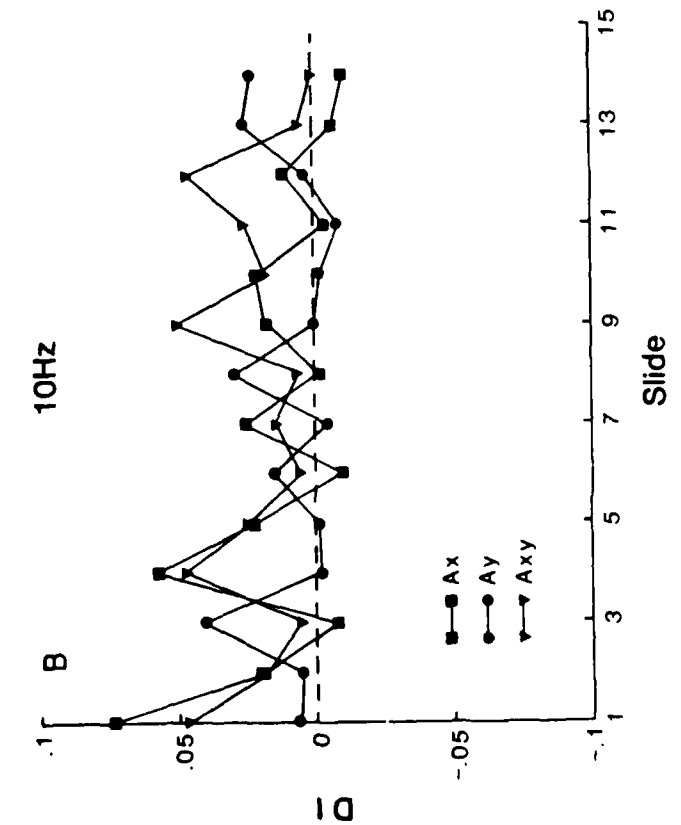
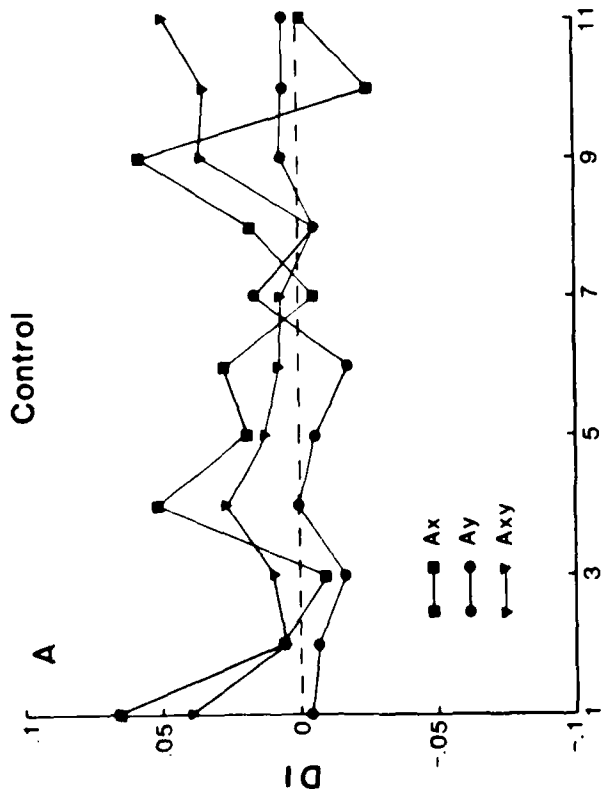


Figure 4

Figure 5



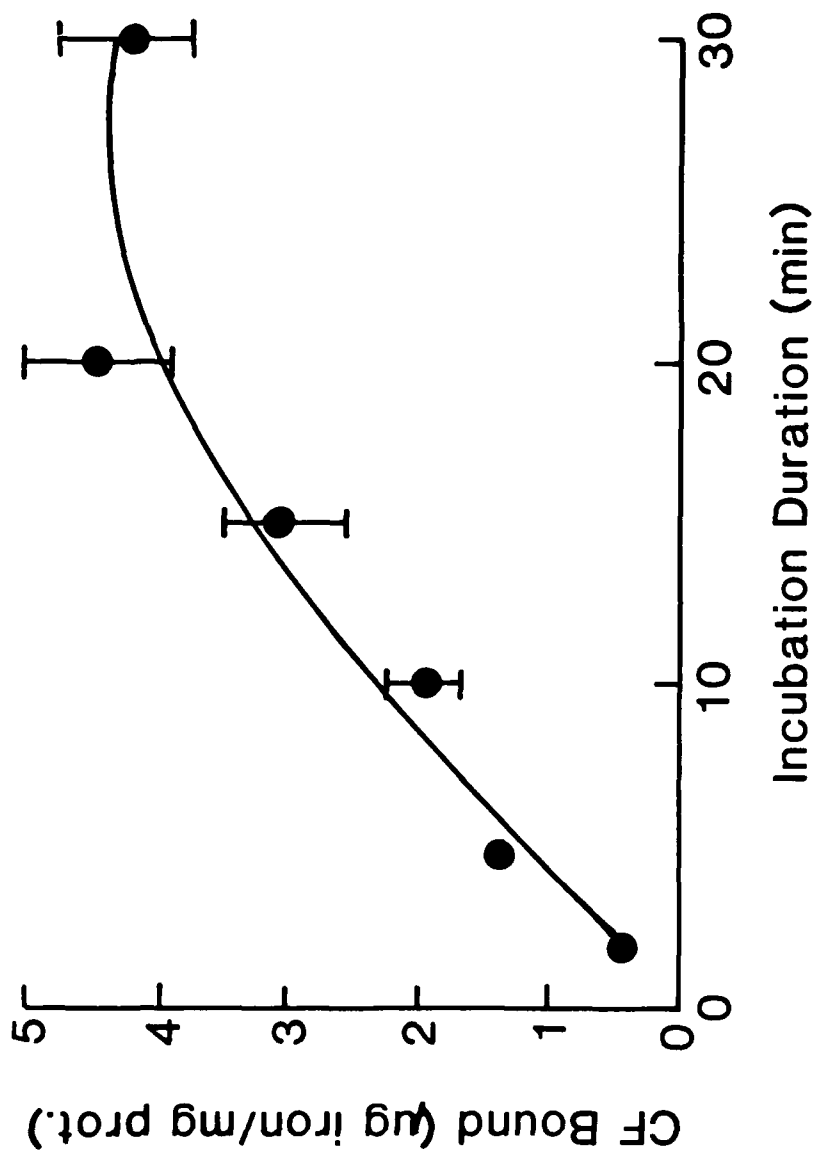


Figure 6

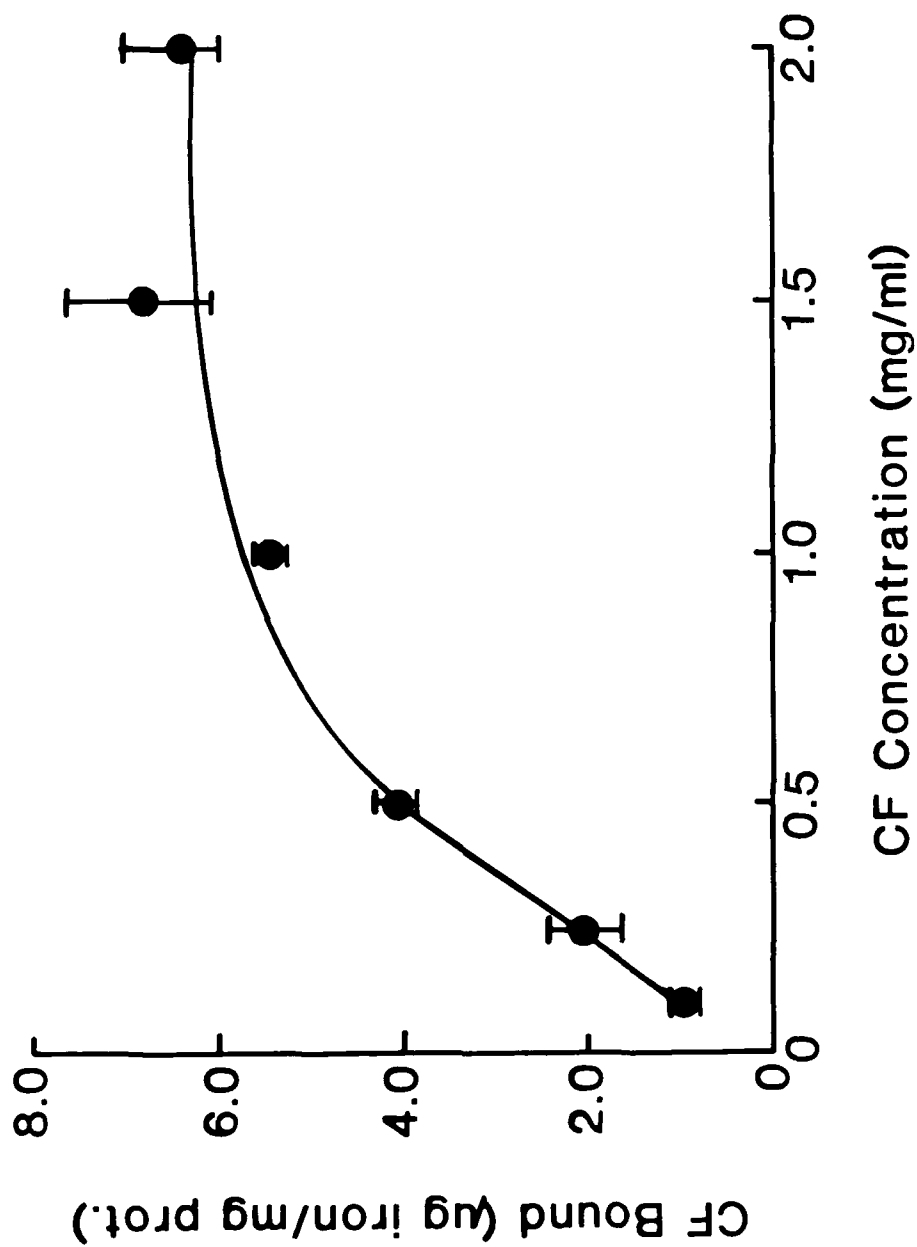


Figure 7



Figure 8

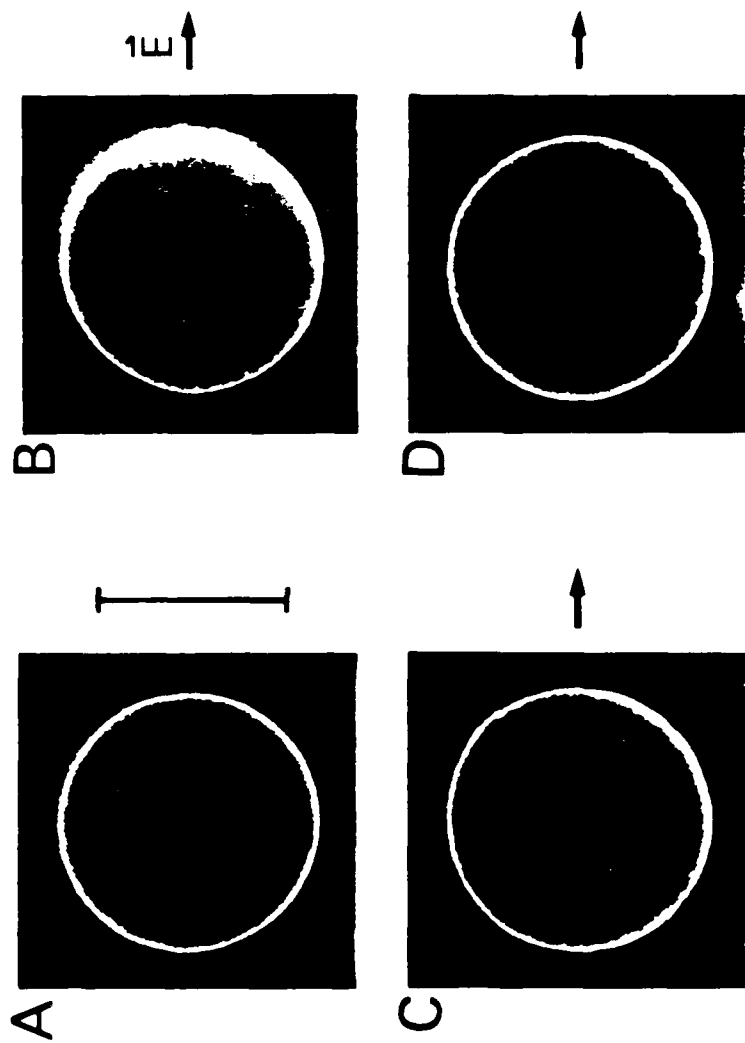


Figure 9

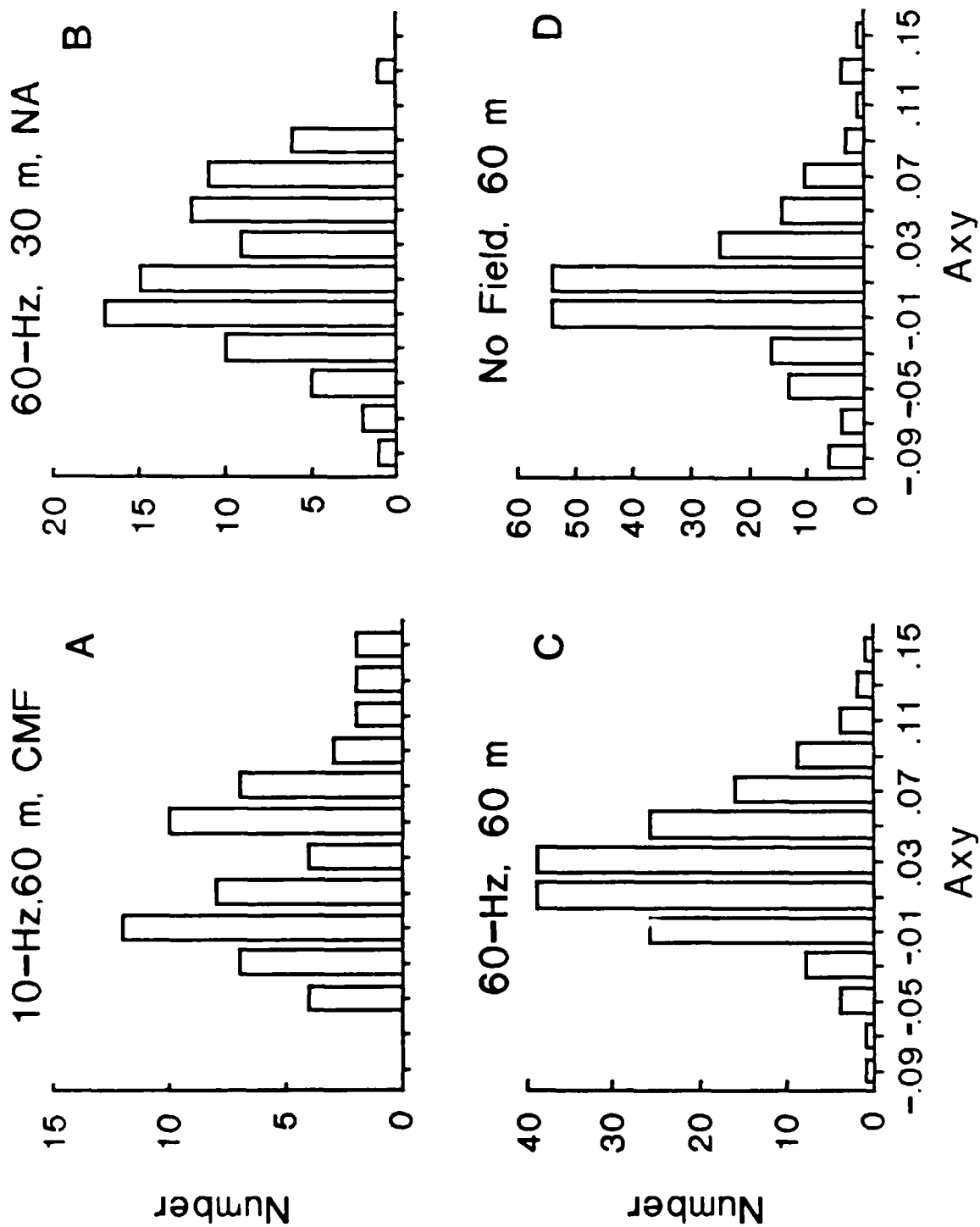


Figure 10

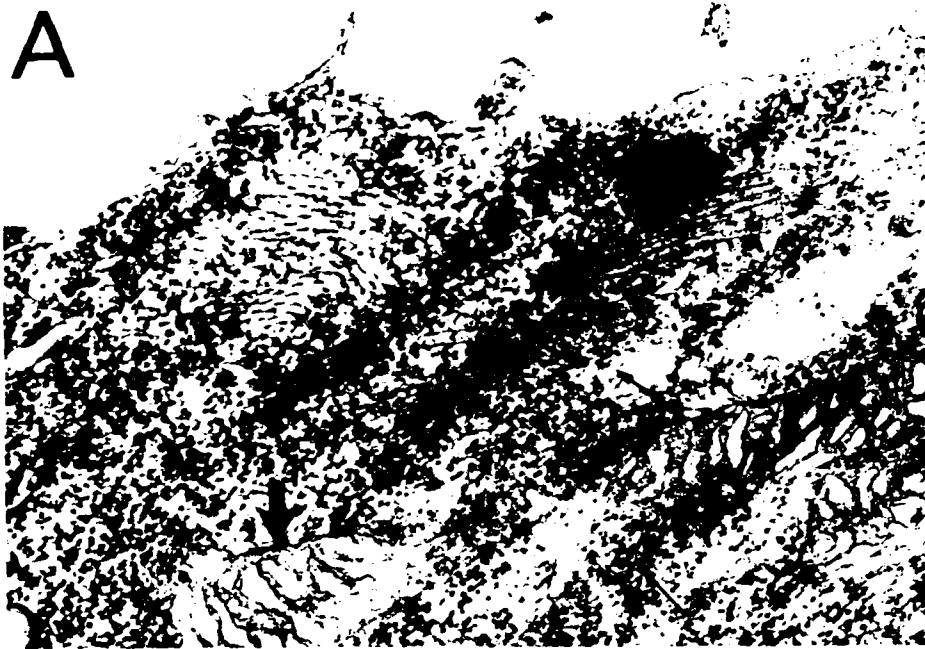


Figure 8

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