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Natural Electrical RF Oscillation from Cells

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Abstract

Electrical oscillatory rf phenomena are present during the division of cells. These were examined by studying the attraction of cells for polarizable powders. They are understood to occur by a process termed microdielectrophoresis (μ-DEP), the motion induced by a nonuniform electric field acting on a polarizable body. The suggestion that an electrical oscillatory aspect may also be involved in the "contact" or density inhibition of cell division and the mechanisms that may cause invasiveness of oncogenic cells are theoretically explored (i.e., changes in either the power level or the frequency of the oscillatory phenomena associated with cell division, or in the degree of electrical insulation of the cell from electrical damping by nearby cells). A number of experiments to test this hypothesis are suggested.

Key Words: Oscillatory rf; polarizable powders; microdielectrophoresis; density inhibition of cell division; invasiveness of oncogenic cells.

Introduction

By observing the attraction of cells for polarizable and nonpolarizable powders, it is evident that certain actively dividing cells emit radiofrequency electric fields. (Pohl, 1979, 1980a, 1980b) Jaffe and co-workers (Weisenseel *et al.,* 1975; Jaffe and Nuccitelli, 1974, 1977; Nuccitelli and Jaffe, 1975; Nuccitelli, 1977) have found static fields associated with growing cells of lily pollen and fucoid ova. The evidence for the presence of such electromagnetic outputs from reproducing cells is at present limited mainly to that for mouse tissue cells with limited data on yeast and on *bacillus cereus,* but it is of interest to ask if the presence of electrical oscillations during the division cycle is a universal phenomenon to cells. If this can be shown to be the case, it may also suggest a link to understanding wound, fetal, and oncogenic growth for the following reasons.

Normal cells exhibit "contact or density inhibition" of growth; fetal and

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oncogenic cells do not (Busch, 1977). The experiments cited above (Pohl, 1979, 1980a, 1980b) suggest that at least mouse cells and probably also yeast and bacterial cells must oscillate during division. If so, then one aspect of the inhibition of multiplication could be that electrical oscillations associated with mitosis are energetically damped by the presence of neighboring cells, but could proceed if surrounded by mostly extracellular medium. This suggests that one or more types of cancer cells, for example, may exploit this aspect of the mitotic process. Cancer cells are characterized by the pathologist by their ability to invade and metastasize (Fig. 1). In the following sections we shall look at the evidence for these suggestions.

The term "contact or density inhibition" has been used by cell biologists to describe arrested cell division at high cell density and has a number of meanings. When cultures of fibroblasts, for example, are examined with the aid of time-lapse photography, one sees that the cells are in continual motion. This motion is greatest at those parts of the cell which are not in "contact" with other cells (Ambercrombie and Ambrose, 1958). "Contact" between these cells appears to inhibit movement at the immediate surfaces. Cellular contact can also bring about cellular adhesion. Some cells that are derived from malignant tumors of connective tissue show neither adhesion nor "contact" inhibition of motion. Szent-Györgyi (1978) has called attention to the role of the lowered degrees of cohesive forces probably present in cancer cells. Laki and Ladik (1976), for example, using quantum mechanical arguments, indicated that the cohesive forces depend strongly upon electronic desaturation in proteins. It is worth stressing, therefore, that we are emphasizing here neither the role of adhesion nor the role of motional "contact inhibition," both of which may indeed be important factors that help differentiate normal from cancer cells. Instead, we are emphasizing here that

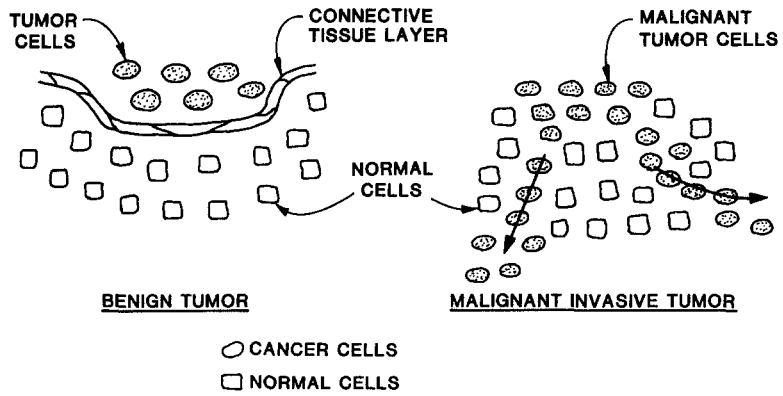

Fig. 1. Diagram showing the difference between a benign and a malignant invasive tumor.

aspect of "contact or density inhibition" relative to cell division (Busch, 1977) and its associated electrical oscillations. The term "contact inhibitions" as normally used by the cell biologist does not necessarily imply that physical contact of the cells is involved, only that close proximity of the cells is involved. Like the term "guinea pig" used to refer to an animal which is not a pig and does not come from Guinea, the term "contact inhibition" is used in connotation rather than in denotation. As applied to fetal, wound-healing, and cancer growth, the meaning is clear.

The evidence for the presence of electrical oscillations in reproducing cells is reasonably direct (Pohl, 1979, 1980a, 1980b) and is based, as noted earlier, upon the observation of the dielectrophoretic attraction of polarizable particles to such cells. Dielectrophoresis (Pohl, 1978), the motion of *neutral* bodies induced by the action of a nonuniform electric field, is a useful phenomenon distinguished from electrophoresis, which is merely the motion of charged bodies induced by an electric field. Dielectrophoresis (DEP) can be considered as resulting from the action of a field to polarize the particle, followed by the pull of the field upon the two regions of equal charge in the particle. Since the field is nonuniform, the pull is greater at one end than the other, and a net force upon the particle results, normally toward the region of higher field intensity. The effect varies as the gradient of the square of the field (E^2) and is, therefore, independent of the absolute direction of the field. Unlike electrophoresis, it can be used effectively in ac fields. Since the polarizability of many systems, especially biological ones, varies considerably with the frequency, a spectrum of responses over a frequency range is possible with DEP, whereas, with few exceptions, only dc effects are useful in electrophoresis. As a result, the DEP of small particles can be used to probe the presence and magnitudes of ac fields in small regions, such as near living cells.

The very short-range electric fields of oscillatory character existing about cells in the reproductive state were detected (Pohl, 1979, 1980a, 1980b) by the dielectrophoretic (DEP) attraction of tiny and easily polarizable particles ($BaTiO_3$ or $NaNbO_3$) as compared with that for the much less readily polarizable ($BaSO_4$ or SiO_2) particles of similar size range. The tests were done mostly on mammalian (murine) cells from either normal (L-cells) or fetal cells that were mostly fibroblasts, or on ascites (cancer) stock. It was observed that rather more (about twice) of the polar particles than of the nonpolar particles were held to the cell surface by actively dividing cells when these were compared with results on cells in stasis (confluent culture). From this behavior, and from the observation that very dilute NaCl or KCl (1 mM or less) could mask and prevent (Pohl, 1980b) the effect, it is evident, or at least simplest to assume, that a chemical specificity for the particular particle surfaces used is *not* a probable cause of the observed behaviors. A further

experimental evidence that the attraction preference for polar over nonpolar particles is a gentle physical one rather than a chemically based one lies in the observation that most of the polar particles attracted to the cells remain in Brownian motion (Pohl, 1979, 1980a, 1980b). It is also very probable that oscillatory electric fields are generated by cells in the reproductive cycle. The frequency of the oscillation in the case of mouse sarcoma ascites cells was judged to be on the order of magnitde of 5 to 1000 KHz, and to be at least 100 V/cm in field strength at the cell surface (Pohl, 1980b).

The occurrence of electrically detectable oscillations in living systems is well documented in other connections (Treherne *et al.*, 1979; Berridge and Rapp, 1979; Rapp, 1979), although not thus far with respect to cell division. Certainly the first biological oscillator system to come to notice was the heart. Long ago, Harvey had called attention to the ability of even small sections of eel heart to continue to contract rhythmically. Early experimenters seem to have associated periodic behavior as being in some sense pathological. The past quarter century has seen a major revision in our view of cyclic chemical and biological phenomena.

If heart muscle has intrinsic oscillations, can a similar process occur on a molecular or cellular level? Early reports of even chemical reactions of periodic nature were largely ignored (Bray, 1921; Hirniak, 1910; Lotka, 1910, 1920). It had been commonly held that all chemically reacting systems would evolve to a steady state, with the components distributed uniformly as to both time and space. This apparent contradiction was cleared up only after several workers (Schrödinger, 1945; Prigogine, 1961) had recognized and stressed the essential distinction between thermodynamically closed and open systems. The relevance of this distinction concerns the production of entropy. The prevalent and historic view had concerned itself with closed systmes. In thermodynamically open systems, the net production of entropy is composed of two parts: (1) the production of entropy within the system, and (2) the exchange of entropy between the system and its surrounding. The latter entropy flow can be either inward or outward, positive or negative. In a thermodynamically open system the outward flow can more than compensate for local entropy generation. Just such an exchange of entropy to the outside is necessary for oscillations to occur, but may be very difficult to quantitate in biological systems.

Still another distinction is recognized as important for a thermodynamic understanding of oscillatory reactions such as those in cells, and that is the distinction between steady state and equilibrium. Prigogine and Balescu (1955, 1956) showed that oscillations are improbable in regions near an equilibrium state, even when rather general requirements (such as the validity of the linear approximations for the Onsager relations) are met. If, however, a given steady state is sufficiently far removed from equilibrium, it

is possible for a chemically driven system to oscillate about that state for an indefinite period. This can, of course, occur without violation of thermodynamic laws (Prigogine, 1978). The early theory (Prigogine and Balescu, 1956) was supported by the experiments of Belousov (1958) showing that the oxidation of citric acid by bromate ion did not proceed to equilibrium monotonically, but instead oscillated between a colorless and a yellow condition. From this grew a wider range of thermodynamic, chemical, and biological studies such as has been recently reviewed (Treherne *et al.*, 1979; Berridge and Rapp, 1979; Rapp, 1979; Prigogine, 1978). Whereas it had been supposed that oscillatory behavior was the sole property of an elite class of "excitable" cells, it is now recognized that oscillatory behavior can be observed in a wide range of cell types. It is our present purpose to suggest that it is a basic property of all cells while dividing.

The number of biological systems for which oscillatory behavior has been observed is quite large (Treherne *et al.*, 1979; Berridge and Rapp, 1979; Rapp, 1979). It ranges for systems which consist of whole organisms from periods of months and even years, to those of smooth muscle (10 to 10^4 sec), to peristalsis (1 to 10 sec), respiration (0.3 to 3 sec), heart (0.03 to 10 sec), and nervous action (10^{-3} to 1 sec). The shortest period so far observed in such systems is about 1 msec which, it might be noted, is that associated with oscillations in a *single* cell. In the case of the oscillations attributed to reproductive processes and detected by microdielectrophoresis (Pohl, 1979, 1980a), the period is not precisely known as yet, but appears to lie in the range of 10^{-3} to 10^{-7} sec, as can be judged from the effect of the conductivity of the support medium (Pohl, 1980a) in masking the field of the cells extending out to the polar particles in micro DEP. It is, thus, apparently somewhat faster than previously observed signals. This may help explain why it has not been previously seen by standard techniques. It is also clear that the previously known oscillatory biological systems can be accounted for by assuming that they are oscillatory chemical reactions. It may be difficult to apply this chemical interpretation to the presumably faster cellular division oscillatory phenemenon, and it may be necessary to turn instead to the ideas of Szent-Györgyi (1978) and Fröhlich (1963, 1973) which are more physicochemical in nature.

Some years ago, Szent-Györgyi (1941) initiated the idea that solid state physics principles in electronic terms could be applied to biology. He suggested then that conduction bands existed in assemblies of protein molecules. That electrons, as such, can traverse the living membrane was demonstrated, for example, in the cases of crustacean cuticle (Digby, 1965), and of the tick salivary gland (Pohl and Sauer, 1978). Fröhlich has applied the kinetics of lightly coupled dipole oscillators regarded as bosons to assemblies of (chemically) driven oscillators, and found from his analysis that at a

certain minimum power input to these dipolar oscillators they would oscillate collectively, and that the fundamental mode was favored (Fröhlich, 1968, 1973). This is reminiscent of the Fermi-Pasta-Ulam problem (Fermi *et al.*, 1965). There, it was observed that lightly but nonlinearly coupled oscillators did not "thermalize" in accord with the ergodic hypothesis, but instead tended to remain in the lowest possible normal modes. This astounding result has since led to extensive mathematical studies of nonlinear systems, including solitary waves and solitons (Scott *et al.*, 1973; Moser, 1979). At this point the analogy of a laser is helpful. In the laser, a certain minimum power level is required to evoke a steady state of lasing. Too little power input to the system (or too much) leaves the system in a nonlasing state. The cellular system that is to oscillate can be considered, in this model, to be similar in that a certain minimum rate of energy input or power level is required for it to reach the oscillatory state (Pohl, 1980a; Fröhlich, 1968, 1973). Efforts should be made to test the present model directly by the use of sensitive radio equipment to look for the intensity, frequency (range), and the extent of the electromagnetic fields about lone cells. In the following sections we describe several additional experiments which confirm that cells, especially those in the rapidly dividing state, emit oscillatory electromagnetic fields.

Experimental

In this section we describe the preparation of the suspensions of the high-dielectric-constant and low-dielectric-constant ("polar" and "nonpolar") powders with cells and the methods for determining the microdielectrophoresis. For preparing powder suspensions, the pure powders (e.g., $BaTiO_3$, $BaSO_4$, $NaNbO_3$, SiO_2) were ground in a mortar and pestle under ca. 10 ml water containing a few drops of liquid detergent (Joy). The product was diluted with more of this liquid to 70 ml and poured into a Petri dish to form a layer 11 mm deep. This was let stand for 5 min and the supernate then carefully decanted into a second Petri dish and let stand for 10 min before pouring off and discarding the supernatant liquid containing the fines. The residual powder in the second dish was now of a rather narrow size range. It was collected and provisionally labeled "2-μ $BaTiO_3$," etc. as appropriate. This was then repeatedly (5×) spun down in a centrifuge and rinsed with deionized 0.25 M sucrose in which 0.1% by weight of soluble (potato) starch had been dissolved (S/S) to aid in stabilization of the powder dispersions.

The powder materials used were: $BaTiO_3$, Alpha Products, 99.99% "2-μ" grade powder; $BaSO_4$, "certified" grade, 2-μ nominal size, Fisher Scientific Co.; $NaNbO_3$ powder, kindly supplied by Dr. P. C. Held, Ceramics Dept., University of Illinois, Urbana, Illinois; SiO_2, amorphous powder,

Imsil-A-15, Illinois Minerals Co., Cairo, Illinois. In passing we should note that solutions of 0.25 M sucrose containing 1% soluble potato starch proved to be very difficult to deionize by passage through ion exchange resins of the mixed bed type (Rohm and Haas MB-3) but that solutions containing the 0.1% starch proved tractable.

The concentrated suspensions of the purified and size-graded powders were shaken with 4-mm-diameter Pyrex glass balls for 1 min in a "Wig-L-Bug" shaker (Crescent Dental Manufacturing Co.) immediately before use with cell preparations.

Cells

Murine fetal fibroblasts were obtained from freshly prepared cultures. The plasticware was purchased sterile. All dissection equipment and glassware were autoclaved at 121°C for 20 min to destroy microbial contamination. All cell culture media were warmed to 25–37°C prior to use except the trypsin–EDTA solution, which was used at 4°C. A 15-day gravid Swiss-Webster mouse (Timco, Houston, TEX) was killed using cervical dislocation and placed beneath a BioQuest cell culture hood. The abdomen was wiped with 70% ethanol and the uteri dissected free. The embryos were freed from the uterus and extraembryonic membranes in a 100 × 20 mm Petri dish containing Hank's balanced salt solution (GIBCO, Long Island, New York) with 100 units/ml penicillin–100 μ/ml streptomycin (GIBCO) and 2.5 μ/ml Fungizone (ISI Biologicals, Cary, ILL), then beheaded, eviscerated, and exsanguinated. The bodies of the embryos were transferred to another 100 × 20 mm Petri dish containing Hank's balanced salt solution, Ca^{++} and Mg^{++} free (GIBCO), and minced into 1–2 mm pieces using razor blades held by forceps. The minced tissue was rinsed twice with Hank's balanced salt solution, Ca^{++} and Mg^{++} free. After the second rinse the tissue was placed in a 125-ml Erlynmeyer flask with stir bar containing 25 ml of Hank's balanced salt solution, Ca^{++} and Mg^{++} free, with 0.5 g/liter trypsin and 0.2 g/liter EDTA (GIBCO). The solution was gently agitated until it appeared turbid (about 15 min). Approximately 2 ml of cell suspension was plated onto 60 × 15 mm Petri dishes to achieve an initial inoculation density of roughly 50% as determined by a Nikon inverted phase contrast microscope. After the cells were allowed to settle for 2 min, each Petri dish was flooded with 15–20 ml of Leibovitz's L-15 medium (with L-glutamine) (GIBCO) containing 10% calf serum (GIBCO), 60 units/ml penicillin–60 μg/ml streptomycin, and 1.5 μg/ml Fungizone. These primary murine embryo cultures were incubated for 12–24 h at 37°C, after which time the culture medium was changed to remove unattached cells. The cultures were allowed to grow for an additional 6 days at 37°C.

In order to assay the microdielectrophoresis of polar or nonpolar particles with these attached fetal cells, the cultures were rinsed four times with 2 to 3 ml of 0.25 M sucrose solution containing 0.2% poly(vinylpyrrolidone) as an aid to particle dispersal. This solution (S/P) was deionized and had a resistivity of 285 kΩ-cm. The cell culture was then flooded with 2 ml of the S/P solution containing added $BaTiO_3$ or $BaSO_4$ particles, and rotated at 1 rps at a 15° angle to the horizontal for 3 min. The liquid was then gently poured off and replaced by 2 ml of the S/P solution. Counting of the cell-particle distributions was then done using a Wild inverted cell culture microscope (M-40) with a Nikon AFM camera attachment. The cells were photographed at 100× in phase and in bright field.

Ascites tumor cells (Sarcoma 180) were obtained from the peritoneal fluid of Swiss mice grown by E. M. Hodnett. Then 2 ml of the fluid was centrifuged for 10 sec at 500 *g* in 1.5-ml plastic centrifuge tubes to remove detritus, then decanted off. The supernate was then centrifuged for 1.5 min at 2500 *g* to sediment the ascites cells. The supernate was discarded and the cells were taken up in two portions, each one in 2 ml of the deionized 0.25 M sucrose containing 0.1% soluble starch (S/S), and again centrifuged; the supernate was removed and the cells again taken up in the deionized S/S. The latter process was repeated four times to increase the resistivity of the suspension to above some 200,000 Ω-cm so as to permit maximal dielectrophoresis.

Ready assay was sought for the microdielectrophoresis occurring between cells and particles. After some search a procedure was devised for allowing the cells and powder to be in contact for a definite time and then stopping the action in a manner to permit viewing and counting.

The mixtures of powder and cells were prepared from 1 ml of the S/S solution, ca. 50 μl of the above cell suspension, and appropriate amount (usually 2.5 to 100 μl) of the powder suspension to provide a useful ratio of cells to particles in the suspension, for later counting. Slides of the mixture of cells and particles were prepared using flat (300 μm i.d.) capillary "microslides" (Vitro Dynamics, Inc., Rockaway, New Jersey 07866) for viewing at 400×. Into the freshly prepared cell–particle mixture, sitting in a 1.5-ml test tube, the microslide was dipped momentarily to sample the suspension at precisely determined times. This permitted cells and particles to freely interact for a known period. The microslide was then pulled out and laid flat. The cells and particles shortly fell through the thin layer (0.1 to 0.3 mm) and rested on the bottom on the microslide ready for counting.

The particular method used with mouse "L" fibroblasts and ascites cells comprised the use of a microscope at 430× and an eyepiece with a graticule having squares 12 μm (in the field) on a side. The microslide was then moved until a cell was centered in a square. The number of particles associated with

the cell, n, and the number of free particles, p, lying in the nine squares containing and abutting the cell were counted. The ratios n/p for each of the numerous cells in the total count was then obtained.

Bacillus cereus. Cell preparation was similar to that for *Bacillus megatherium* developed by Hunter-Szybalski *et al.* (Hunter-Szybolska *et al.*, 1956; Szybolska and Hunter; Szybolska, 1955). Synchrony of growth was obtained by chilling the logarithmically growing population for 30 min to 15°C, then returning to constant 34°C, which is an optimum temperature for growth. A 40-min lag, in the case of *B. megatherium,* is succeeded by several cycles of usual length corresponding to the normal bacterial generation time, about 32 to 38 min. Each cycle, according to optical density curves we obtain with the *B. cereus,* appears to be composed of a sudden duplication followed by a relatively stationary period. Cold appears to arrest division or perhaps to accumulate the nuclei in a condensed state, which in the phased population appears only just following division. It has been described (Zeuthen, 1958) as "metaphase-like." Reincubation at 34°C is reported to cause rapid reconstitution of "sister chromosomes," and within 30 to 40 min the nuclear structure resembles the "filamentous chromosomes" of the pre-chilling period (Zeuthen, 1958), from which the organism proceeds into the condensed state, after division of the nucleus and of the cell.

Pure stock culture was made from streak plates grown on nutrient agar slants. Both cultures were grown in nutrient broth (Difco). Growth curves obtained on the latter showed the expected growth synchrony population curves as judged by optical density studies.

Assay of the microdielectrophoresis of the $BaTiO_3$ and $BaSO_4$ particles (2 μm average diameter) was done by mixing the particles and cells in 1.5-ml tubes. Into the freshly stirred suspension of cells and powder particles (i.e., either $BaTiO_3$ or $BaSO_4$ was plunged a 0.3-mm path length "microslide" (#3530 from Vitro Dynamics, Inc., 114 Beach St. Rockaway, New Jersey 07866) so as to fill the microslide. The microslides were held in the suspension precisely vertically for 3 min, pulled out and laid flat on a microscope slide for settling of the cell–particle suspensions, and then photographed at 400 $\times$ under a microscope, for subsequent analysis. The photomicroscope, with phase contrast (Diavert), was made available through the courtesy of H. Muller, Leitz Corp. The results are tabulated in Table I.

Results and Discussion

The accumulation of particles on the cells was observed as described earlier, and the number of cells per unit volume, c, the number of particles associated with cells per unit volume, n, and the number of free particles per

Table I. Particle-Gathering Ability of Various Cells for Polar and Nonpolar Particles

Cell type	Medium	Resistivity of medium (Ω-cm)	Particle type	Particle gathering ability, k ($k = n/cpt$)	Ratio of k (polar) to k (nonpolar)
Mouse, fetal fibroblasts, rapidly dividing	0.25 M sucrose + 0.01% agar	1.4×10^5	$BaTiO_3$	$0.0094 \pm 0.0032(4)$	1.9
Same as above	Same as above	1.4×10^5	$BaSO_4$	$0.0050 \pm 0.00055(4)$	
Mouse, fetal fibroblasts, confluent	0.25 M sucrose + 0.01% agar, as above	1.4×10^5	$BaSO_4$	$0.0011 \pm 0.00005(2)$	—
Mouse, fetal fibroblasts, rapidly dividing	0.25 M sucrose and 2% poly(vinyl pyrrolidone)	2.8×10^5	$BaTiO_3$	$0.137 \pm 0.058(5)$	2.0
Same as above	Same as above	2.8×10^5	$BaSO_4$	$0.066 \pm 0.025(5)$	
Mouse, fetal fibroblasts, rapidly dividing	0.25 M sucrose and 2% PVP	2.8×10^5	$BaTiO_3$	$0.073 \pm 0.042(5)$	2.0
Same as above	Same as above	2.8×10^5	$BaSO_4$	$0.036 \pm 0.010(5)$	
Mouse, ascites	0.25 M sucrose and 0.01% agar	3.0×10^5	$BaTiO_3$	$5.2 \times 10^{-4} \pm 1.35 \times 10^{-4}(4)$	2.2
Same as above	Same as above	3.0×10^5	$BaSO_4$	$2.4 \times 10^{-4} \pm 2.2 \times 10^{-5}(4)$	
Mouse, ascites	0.25 M sucrose and 1% potato starch	22×10^3	$BaTiO_3$	$0.0082 \pm 0.016(2)$	2.9
Same as above	Same as above	22×10^3	$BaSO_4$	$0.0029 \pm 0.0005(2)$	

Mouse, ascites	Same as above	30×10^3	$BaTiO_3$	$0.035 \pm 0.012(11)$	1.05
Same as above	Same as above	30×10^3	$BaSO_4$	$0.033 \pm 0.014(11)$	
Mouse, ascites	0.25 M sucrose and 1% potato starch	30×10^3	$BaTiO_3$	$0.012 \pm 0.0092(5)$	1.2
Same as above	Same as above	30×10^3	$BaSO_4$	$0.010 \pm 0.0021(5)$	
Mouse, ascites	0.25 M sucrose and 0.1% potato starch	2.8×10^5	$NaNbO_3$	0.21 ± 0.037	2.7
Same as above	Same as above	2.8×10^5	SiO_2	0.078 ± 0.026	
Bacillus cereus, early phase	Water	2.5×10^5	$BaTiO_3$	$0.108 \pm 0.040(6)$	6.0
Bacillus cereus, early phase	Water	2.5×10^5	$BaSO_4$	$0.018 \pm 0.012(3)$	
Bacillus cereus, early phase	Water	2.5×10^5	$BaTiO_3$	$0.028 \pm 0.0012(6)$	3.5
Bacillus cereus, early phase	Water	2.5×10^5	$BaSO_4$	$0.0079 \pm 0.00024(9)$	
Bacillus cereus, early phase	Water	2.5×10^5	$BaTiO_3$	$0.019 \pm 0.046(8)$	12
Bacillus cereus, early phase	Water	2.5×10^5	$BaSO_4$	$0.0016 \pm 0.0009(10)$	

unit volume, p, determined. There are a number of ways in which these data can be analyzed, depending upon the assumptions made for the kinetics of particle collection by cells.

One simple approach is to assume that each particle that hits a cell stays there ("black hole" model). Another is to assume that as particles arrive, they block the further accumulation of particles upon the cell (fillable black hole). Another model would focus upon the known tendency of particles in an electric field to amass other particles to themselves by a process known as "mutual dielectrophoresis" (Pohl, 1978). There are obvious combinations of these processes, and doubtless still others which merit attention. We can summarize several of these approaches as follows: The accumulation of particles by the suspended cells, whether by the simple act of diffusive and sticky collections, or under the attractive guidance of dielectrohporetic force upon the particles as they experience that ac field about the cell, can be regarded as a series of (loosely) related events, viz.

$$\text{Cell} + \text{particles} = \text{agglomerate}$$

$$\text{C} + \text{P} = \text{CP}_1$$

$$\text{CP}_1 + \text{P} = \text{CP}_2$$

$$\cdots\cdots\cdots\cdots\cdots$$

$$\text{CP}_{n-1} + \text{P} \overset{k}{=} \text{CP}_n$$

For a simple sequential process ("black hole") where a cell can hold an unlimited number of particles, we have

$$dn/dt = k_0 cp \tag{1}$$

where k_0 is a constant characteristic of the specific rate of particle accumulation.

Again, for cells in the second case, we may roughly describe saturation of the cell surface by including a saturation term, viz.

$$dn/dt = kcp(1 - n/p_0) \tag{2}$$

where

$$p_0 = n + p \tag{3}$$

is the original concentration of free particles before the cells acted to accumulate them.

For particle accumulation by purely particle-to-particle interaction, after the first particle has been collected by the cell field and has induced a field in the particle, one could expect a mutual dielectrophoresis, in the early

stages at least, obeying kinetics of the sort describable by

$$dn/dt = knp \tag{4}$$

In the integrated form, these become
(a)["black hole" model following Eq. (1)]

$$n = p_0[1 - \exp(-k_0 ct)] \tag{5}$$

or

$$k_0 = \ln[(p + n)/p)]/ct) \tag{6}$$

(b) [fillable "black hole" model, following Eq. (2)]

$$n = p_0 kct/(1 + kct) \tag{7}$$

or

$$k = n/(cpt) \tag{8}$$

(c) [mutual dielectrophoresis of particles onto particles already on cells following Eq. (4)].

In this case the integration must begin with the assumption that there is just one particle upon the cell and that it continues, i.e., $n_0 = c$.

$$\ln\{[(p_0 - c)/c][n/(p_0 - n)]\} = p_0 k_n t \tag{9}$$

or

$$k_n = (p + n)^{-1} t^{-1} \ln\{[(p + n - c)/c](n/p)\} \tag{10}$$

Inspection of the size of the standard deviations of the data analyzed according to Eqs. (6) or (8) indicated a slightly larger deviation accompanied the latter, but the former offers difficulties in averaging, so the data are presented in terms of the rate, k, for Eqs. (2) and (8). We regard the use of Eq. (8) as temporary and provisional pending further investigation.

The accumulation of particles by the mouse fetal fibroblasts that are rapidly dividing is seen to favor the pickup of high-dielectric-constant particles. The same is observed to be true for rapidly dividing ascites mouse cells. The early-phase *Bacillus cereus* also shows a preference for amassing of the more polarizable particles. We conclude that in the cases observed here, i.e., mouse fetal fibroblasts, mouse ascites tumor cells, and *B. cereus* (early phase), rapidly dividing cells exert a preferential attraction for the more polarizable particles, the $BaTiO_3$ (dielectric constant about 2000), over that of the less polarizable particles, the $BaSO_4$ (dielectric constant about 12). Similar remarks apply to the case of the preference of murine ascites cells for the high-dielectric-constant material (ca. 330) $NaNbO_3$ when compared with

the pickup of the low-dielectric-constant material (ca. 4) SiO_2. The similar actions of cells for the very different chemicals $BaTiO_3$ and $NaNbO_3$, and for $BaSO_4$ and SiO_2, suggests that the interactions observed are more related to the polarizabilities (dielectric constants) than to the chemical surfaces, etc.

Let us return now to the matter of "contact" or density inhibition of cellular reproduction, and the postulated necessity for electromagnetic oscillations during the mitotic cycle. As mentioned, there are numerous possible causes which could contribute to "contact inhibition" of reproduction. Among these are local concentration effects due to lack of nutrient, or an overabundance of metabolic by-products. We suggest that one further aspect of the "contact inhibition" may be the ability of the required electrical oscillations to continue only if the cell is not too closely or critically associated with electrically "lossy" or dissipative material in its surroundings. It is well known that living cells exhibit both a higher dielectric constant and, at certain low frequencies, higher dielectric loss (energy absorption rate) than that of the surrounding fluid (Pohl, 1978; Schumann, 1956; Fricke and Morse, 1925) (plasma, etc.). With this in mind it may be that a cell which is ready in all other respects to begin dividing could be inhibited from oscillating and hence dividing, because its oscillation would be damped out by unfavorable surroundings. Using this scheme, one can postulate at least three ways in which a cell might override the normal electrical inhibition process due to the presence of other (and, therefore, lossy) cells.

(1) Power Level Increase. If the cell has the ability to devote energy at a higher-than-normal rate to its oscillatory system, it could override the power loss occasioned by the dissipative surroundings such as neighboring cells.

(2) Insulation. If the specific cell of interest is immediately at least partially surrounded by a cocoon or other relatively nondissipative coat, this could afford sufficient insulation or decoupling from its neighbors so that the necessary electrical oscillation could arise, and allow cell division to continue (Fig. 2).

(3) Frequency Shift. As indicated in Figs. 3 and 4, the usual course of the dielectric constant and dielectric loss curves for a particular dielectric

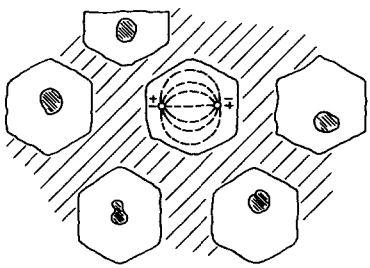

Fig. 2. Diagram of liver cancer (hepatoma) cells of guinea pig (after Dvorak *et al.*) showing fibrous intercellular spacing ("cocoon") and the lines of force as for an electrical oscillator within a cell.

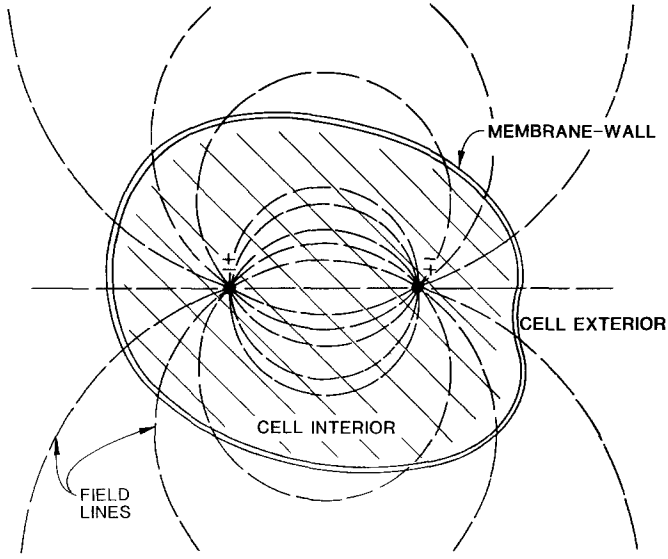

Fig. 3. Electric field around a cell.

material shows a monotonic sharp decrease of the dielectric constant (permittivity) at some characteristic frequency, and rather high peaking of the dielectric loss curve close to that frequency. One could understand the inhibition of cell division for normal cells in terms of having them operate their oscillating systems at or near the critical maximum of the loss curve of neighbor cells. In this manner, the presence of other cells would be highly likely to subdue or damp out their necessary electrical oscillation. On the

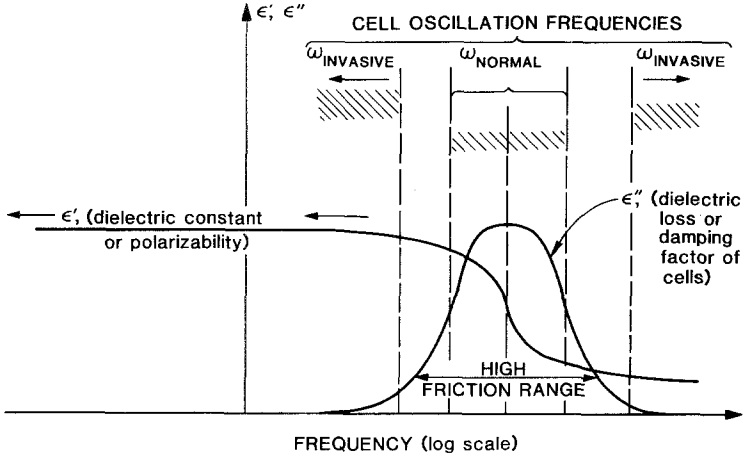

Fig. 4. Dielectric constant and dielectric loss versus frequency.

other hand, a cell which would only minimally be inhibited by the presence of other cells could be understood to have somehow shifted its own electrical (reproductive) oscillation frequency either sufficiently up or down scale from the loss maximum of the medium so as to be essentially decoupled from its surroundings.

One mechanism by which fetal or cancer cells show less contact inhibition of growth than normal cells may well involve one of these three possible mechanisms. In particular, the invasive character of cancer cells could, in some cases, involve such behavior.

This model predicts, for example, that cells bearing a thickish coat of low-loss material would show reduced "contact inhibition" due to electrical dissipative means. This would fit the observations of Landman and co-workers (Landman and Hall, 1963; Ryter and Landman, 1964) who showed that protoplasts of various bacteria were unable to reproduce unless a certain minimal coating had been formed about the protoplasts. Further evidence for this in the case of cancer cells was presented by Dvorak (Dvorak *et al.*, 1979a, 1979b) and collaborators. They showed that in the guinea pig some tumors can insulate themselves from the host animal's defenses by building a "cocoon" of gel-like fibrin about themselves. While the presence of fibrin is known to stimulate blood vessel development, crucial to the tumor support, it is also possible that the cocoon affords the necessary electrical insulation for the cancer cell so that electrical oscillation necessary for the continued reproduction (or invasion) can continue. The cocoon probably also affords some protection from antibody attacks.

The differing dielectric character of cancer cells from that of normal cells was revealed in the remarkable pioneering work of Fricke and Morse (1925). They showed that tumors of the human breast (58 cases) have a higher specific capacity at 20 kHz than that of normal tissue. To our knowledge this work has not been repeated or verified, but it should be.

There are a number of experimental studies on cancer cells which permit interpretation in terms of the hypothesis that electrical oscillations are requisite for cell division. The frequency of those oscillations observed to date (Pohl, 1979, 1980a, 1980b) is quite low (on the order of 10^3 to 10^6 Hz), indicating that large regions of the cell must be involved, and arguing for cytoplasmic rather than just nuclear involvement. Among the various physiological evidences which support this is the fact that when nuclei were removed from normal oocytes and inserted into enucleate oocytes by microsurgery, a perfectly normal frog develops. Similarly, when a nucleus from an inactive but fully differentiated tadpole intestinal cell is inserted into an enucleated oocyte, a perfectly normal frog develops. This demonstrates that tissue differentiation does not remove any of the "information storage bank" from the nucleus of the differentiated intestinal cell of the tadpole. Busch (1977)

concludes from this that the differentiation of virtually all of the cells in the body is, therefore, cytoplasmic. Since this holds true for cancer as well as for other types of cell, the implications are very broad, i.e., that cancer is a *cytoplasmic* disease that develops from inappropriate transcriptions from the information storage bank of the cell nucleus (Busch, 1977). The importance of the cytoplasmic aspect is supported by the fact that, as H. Harris and his colleagues (Gallo, 1977) have shown, fusion of cells of apparent neoplastic origin (having *in vitro* characteristics of neoplastic cells) with presumably normal cells has generally indicated that the malignant state can be suppressed.

Endogenous electric fields from cells are suggested by spectral studies. Webb *et al.* (1977) have shown that the microwave and laser-Raman spectra of human carcinoma cells displayed a splitting not detectable in those of normal cells. Moreover, they showed in preliminary experiments that brief irradiation with certain high-frequency electromagnetic fields would remove the ability of the still viable (baby hamster kidney) cells to form tumors in susceptible animals.

We have mentioned above that there are two possible origins for the growth-associated oscillations: (1) oscillatory chemical reactions, or (2) long-range collective oscillations of synchronized dipole states (Fröhlich, 1968, 1973).

In the case of the oscillatory chemical reactions, it is known that free-radical chain reactions which branch multiply (i.e., one produces three, etc.) are involved (Noyas and Field, 1974; Rabai *et al.*, 1979; Winfree, 1974; Degn, 1972). Such systems will be sensitive to the presence of free-radical inhibitors, etc. We have observed, for example, that the Belousov–Winfree cyclic reaction (Winfree, 1974; Degn, 1972) of bromide, bromine, malonic acid, and ferrous phenanthroline is readily affected by methyl glyoxal, an electron acceptor molecule. The addition of 0.1% methyl glyoxal to the reaction mixture speeds up the cycle time by a factor of 5, reducing the period from 25 to 5 sec. On the other hand, the addition of up to 0.6% ascorbic acid had little effect. Such cyclic reactions could perhaps serve as model systems for studying the effects of chemical agents on the reactions responsible for cellular cyclic reactions. Rabai *et al.* (1979) used ascorbic acid in their study of oscillating reactions. There is the exciting possibility that the chemical reaction waves are linked to and controllable by electric fields, as suggested by the theory of Schmidt and Ortoleva (1979). The phenomenon of mechanically induced stimulation of electromagnetic radiation may be useful in helping observe and correlate this (Pohl, 1980a).

Oscillating electric fields (5 Hz) have recently been shown to affect the rate of DNA synthesis in cartilage cells (Rodan *et al.*, 1978) obtained from the proliferative zone of the tibia epiphyses of 16-day chick embryos. The

effects were completely blocked by micromolar concentrations of verapamil or tetrodotoxin. The findings support the hypothesis that Na^+ and Ca^{++} fluxes aroused by the perturbation serve to trigger DNA synthesis in these cells. There is little doubt that more detailed insight into the charge distributions, dipole moments, and their oscillations in cell membranes would add to the understanding of the precise nature of the electrical events (Pilla, 1974; McLaughlin, 1975) experienced and generated by living cells. Fields which are of very high frequency (10^9–10^{11} Hz) are known to have effects, mainly thermal, on higher animals. These effects are well discussed in recent papers and reviews (McLaughlin, 1975; Cleary, 1979).

There have been a number of elegant studies of the static fields present about single-celled systems. Low-frequency pulses (about one per minute) have been observed in the growing pollen tube (Weisenseel *et al.*, 1975) of the lily *Lilium longiflorum* cv. *Arai.* as it attains a length of circa 1 mm. Jaffe and co-workers conclude (Weisenseel *et al.*, 1975) that here, as in their studies of the fucoid alga *Pelvetia* (Jaffe and Nuccitelli, 1974, 1977; Nuccitelli and Jaffe, 1974, 1975; Nuccitelli, 1977) they always see a current about and through the organism associated with growth. This is a most significant observation.

In considering the effects of externally generated fields upon cells, one must be aware of the limitations imposed by the nature of the medium supporting the cell. These in turn impose severe limitations upon one's ability to impress effective fields within the cells themselves. The specific power w produced by Joule heating in a volume to which a field E is applied and which has a specific resistivity p is

$$w = E^2/p$$

If we take a power density of about 0.01 W cm^3 as a reasonable and safe limit for our circumstance, then we can see that it will be difficult to safely apply a field greater than a few volts per centimeter in blood or sera with specific resistivities of about 100 Ω-cm, or in tissue typically (Ackerson, 1962) with specific resistivities of about 400 to 1000 Ω-cm. We note then that we can safely and briefly apply only a few V cm^{-1}, whereas the fields internal to cells may be much larger. The field across the cell membrane is typically about 100,000 V cm^{-1}. The field strength estimated to arise at the outer surface of growing cells, as determined by microdielectrophoresis measurements (Pohl, 1979, 1980a) using the ferroelectric $BaTiO_3$, were on the order of 100 V cm^{-1}. These calculations indicated that for the application of external fields to cells to have a dramatic (and positive, not negative) effect, one would need to work with cells suspended in media of low conductivity, otherwise Joule heating and its deleterious effects might mask other effects. That is not to say

that applied electrical fields at the necessarily low levels for safety in whole tissue would not evoke discernible effects, but only to emphasize that at these necessarily low levels of applied field, the effects might require careful analysis to recognize.

The postulated necessity of electrical oscillations for cellular reproduction and its associated suggesting of a linking of the electrical oscillations with "contact inhibition" of growth can be clarified by some or all of the questions and experiments below.

1. Would the invasiveness of cancer cell into a spongy substrate material be affected by the effective dielectric loss of the spongy substrate? The hypothesis predicts that very lossy material would evoke growth inhibition if the loss peak frequency is near that of the cellular oscillation.

2. What is the relation of the electrical oscillation to the phase of cell growth? G^1, S, G^2, or M?

3. What is the source of the reproductive electrical oscillations? Is it (a) related to an oscillatory chemical reaction cycle, or (b) based upon a more physically derived phenomenon such as that of the Fermi–Pasta–Ulam–Fröhlich type (Pohl, 1980b)?

4. Does electrical oscillation continue if the cell is made to stop in its reproduction cycle, as by use of agents such as colchicine, colcemid, or Nocodazole?

5. Is that growth of compact or confluent cancer cells inhibited by digesting off the "cocoon" with tryspin (or plasminogen)? And restored by recoating as by immunoglobulin and then antibodies bearing gylcoproteins or by producing fibrin?

6. Is the growth of confluent normal cells (e.g., fibroblasts) spurred by their bearing an insulative coating of fibrin or glycoprotein?

7. If a survey of electron micrographs of tissue sections is made, do cancer cells appear to have partial or complete insulative "cocoons" or intercellular regions more often than do normal cells from like origins?

8. Is electrical oscillation necessary (and/or observable) in all types of reproducing cells? Or do some cell types not need to oscillate to reproduce?

9. What is the strength, range, and frequency of the electrical oscillations associated with reproduction?

10. Is there a preferential orientation of the mitotic poles of cell groups? Are they in phase? Is the orientation responsive to externally applied ac fields?

11. Is cellular growth affected by properly (see remarks above) externally applied ac fields?

12. Does the critical frequency, or range thereof, of normal cells differ from that of wound-healing, cancer, or embryonic cells? Via gene *Tu* (Ahuja and Anders, 1977)?

13. Do these critical frequencies (or their ranges) correspond to the frequency of the dielectric loss peaks of normal cellular tissue?

14. During cell divisions from fertilized egg to blastula, is there evident dipole interaction due to the postulated electrical oscillatinos? Or overall organization(s) of the ac fields about the cells?

15. Are the electrical oscillations required for cell division, or are the oscillations simply a result of the division process? The cause and effect relation needs study.

Study of these and related questions can serve to verify the several lines of thought proposed and determine if oscillating electromagnetic phenomena are a ubiguitous characteristic of dividing cells and if overriding of their subsequent interference is an insidious characteristic of invasive cells.

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