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Fröhlich Electromagnetic Radiation from Human Leukocytes: Implications for Leukocyte Adherence Inhibition Test

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The Fröhlich coherent vibrations may be a source of an electromagnetic field generated by living cells in the frequency range from 0·1 to 10 THz. The electromagnetic field may cause the time dependent orientation (i.e. rotation or rocking) of the polar molecules of the ambient liquid medium and may attract them. The attracted molecules move together with the cell and the friction coefficient of the cellular motion, therefore, may depend on the field. The cell-generated electromagnetic field may interact with the surface charge of various solid-state materials causing attractive forces. These interaction attractive forces may be significant in the process of the leukocyte adherence to the surfaces of various materials. The hypothesis presented in this paper assumes that the exposition of leukocytes from immune individuals to antigen causes changes of the Fröhlich coherent vibrations resulting in decrease of the leukocyte adherence observed in the leukocyte adherence inhibition test.

1. Introduction

The leukocyte adherence inhibition (LAI) test is a method detecting cellular immunity (Halliday & Miller, 1972; Holáň *et al.*, 1974). It is based on the observation that in the presence of antigen, the leukocytes from an immune individual manifest decreased adherence to glass or plastic materials. Numerous attempts have been made to use this test for detection of malignat tumors in humans. A review is given by Burger (1979).

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There are long-range and contact hypotheses explaining the adsorption, attachment and subsequent spreading of cells. The long-range hypothesis is based on the balance between long-range electrostatic and dispersive forces (Curtis, 1973). The contact hypothesis assumes polymer-bridging between cell membrane and substrate surface (Maroudas, 1975*a*, *b*), protein adsorption onto the substrate surface, cellular microextensions (microvilli and filopodia) and cellular microexudates (Grinnell, 1978). The requirement for attachment and spreading seems to be substrate surface charge density. Negative surface charges are usually assumed. Spreading as a function of surface charge density peaks around 2–10 negative charges per nm^2 (Maroudas, 1977), but cells will spread equally well on strongly positive surfaces (Maroudas, 1975*a*).

In this paper the Fröhlich coherent vibration hypothesis is used to explain the decrease of the leukocyte adherence observed in the LAI test after exposition of the leukocytes from immune individuals to antigen. The Fröhlich hypothesis assumes that the metabolic energy supply to the polar vibrations in living cells (e.g. to the vibrations in cellular membranes) leads to the condensed (coherent) vibration state with a great energy storage. The electric charge oscillations connected with the vibrations are the source of the electromagnetic field and of the long-range selective interaction between vibrating systems (Fröhlich, 1968*a*, *b*, 1972, 1977, 1978, 1980; Pokorný, 1982*a*). Cell-generated oscillating field results in attractive forces with both positively and negatively charged substrate surfaces if both the mobility and concentration of substrate charge carriers are high enough.

The force field of cellular origin extending at least to a distance of about 8 μm from the outer cell surface was experimentally proved for human erythrocytes from their collision frequency (Rowlands *et al.*, 1981). The energy supply cut off with the zeta potential of the cell unchanged causes the extinction of the force field. Further experimental evidence is given by Rowlands, Sewchand & Enns (1982) and by Sewchand, Roberts & Rowlands (1982). The electromagnetic character of the cell-generated field was proved by Pohl (1980) and by Roy, Braden & Pohl (1981). These experiments were based on the attraction of small dielectric particles by ascites tumour cells (sarcoma 180).

2. Fröhlich Electromagnetic Field Generated by Living Cells

We assume the membrane of the cell to be the source of the high frequency electromagnetic field. The coherent electric dipole vibrations are maintained by the cellular metabolic energy supply. We adopt a spherical dipole layer model of the membrane. The spherical layer is

composed of identical elementary electric dipoles the axes of which are perpendicular to the spherical surface. All the elementary dipoles oscillate in the same phase. The formulae for the electric field radiated by an electric dipole are given (e.g. in Stratton, 1941). Instead of the real permittivity the complex one is used in these formulae, which partially covers the effects of the conductivity on the electromagnetic field generated by the cell. The currents caused by the generated electromagnetic fields are not explicitly involved in the source function integral in the Kirchhoff integration method and screening effect of charges at the boundaries is neglected.

Numerical integration was used to add the contributions of the elementary dipoles located in the area of the spherical surface visible from the point of observation. The computed electric field in the frequency range from 0·1 to 10 THz is given in Fig. 1. Within a distance of about 10 μm

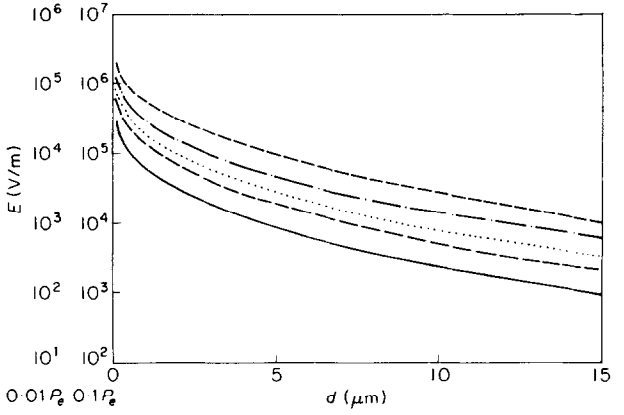


FIG. 1. Logarithmic plot of intensity E of the electric field generated by the living cell against distance d from the outer cell surface. The vertical scales hold for elementary dipole moments $0{\cdot}1P_e$ and $0{\cdot}01P_e$, where $P_e = el$ (e is the elementary charge and l is the thickness of the membrane; we assume $l = 5$ nm). The elementary dipole cross section is 10^{-18} m^2. The frequencies are — — —, 10 THz; — · — · —, 3 THz; · · ·, 1 THz; – – –, 0·3 THz; ——, 0·1 THz. The complex relative permittivity values used in computation are $8{\cdot}5 + j12{\cdot}5$ (for $f = 0{\cdot}1$ THz); $5 + j5$ (0·3 THz); $4{\cdot}5 + j2{\cdot}5$ (1 THz); $3 + j1{\cdot}7$ (3 THz) and $2 + j$ (10 THz).

from the outer surface of the spherical dipole layer there are significant values of the electric field intensity even for dipole moments smaller than those used in Fig. 1. The field may have more than one frequency component (Pokorný, 1982*b*). Besides the dipole layer the multipole layer ought to be considered as well. The generated field may even have a tesseral (zonal) harmonics character—domains on the cellular surface may be formed (Pohl, 1980).

The selective interaction forces between cells (i.e. the attractive and repulsive forces) and their changes are most likely mediated by this electric field (Pokorný, 1980). This field should cause space orientation (rotation or rocking) of polar molecules, e.g. of water. Molecular dipoles are not only orientated but also attracted in the direction of the gradient of the electric field. It follows from Fig. 1 that especially in the vicinity of the cell surface the gradient of the electric field is significant. The living cells ought to have a "cloud" of attracted rocking water molecules around them and move together with this "cloud". This holds especially for the lower frequency fields for which the dipole rotation is fast enough.

The electric field acts on the surface charge of various materials. If the mobility of the charge carriers is high enough (for reasonably high concentration), the surface charge distribution may be changed. This results in the attractive forces between the living cells and the surface of a solid-state material. The mobility of electrons in the surface states of glass or plastic materials is usually high enough in the frequency range below 10 THz.

3. Sedimentation Rate of Leukocytes

An elementary theory of the leukocytes' (peripheral white blood cells) sedimentation in a one-dimensional approximation (e.g. sedimentation in a broad vessel with a flat plane bottom) is presented. Assuming the uniform leukocyte distribution in a liquid medium at the initial time we can calculate the time dependence of the leukocyte sedimentation from the equation

$$m_i \dot{v}_i + f_i' v_i = g(m_i - m_{0i}), \tag{1}$$

where m_i, v_i and f_i' are the mass, velocity and the friction coefficient of the leukocytes, respectively, $\dot{v}_i$ is the time derivative of v_i, g is the gravitational constant and m_{0i} represents the upthrust. The leukocytes are assumed to have a spherical shape. The friction coefficient of a sphere moving in a liquid is given by Stokes's relation $f_i' = 6\pi f_0' R_i$, where R_i is the radius of the sphere and f_0' depends on the viscosity of the liquid. (For a rigid sphere the coefficient f_0' is equal to the viscosity but the dynamic deformation of the spherical cell may decrease its value.) Parameters of the leukocytes of different types are denoted by the index i (i.e. m_i, v_i, f_i', m_{0i} and R_i). From equation (1) we get (after integration $z_i = \int_0^t v_i \, \mathrm{d}t$)

$$z_i = g\frac{m_i - m_{0i}}{f_i'}t + \frac{m_i}{f_i'}\left(v_0 - g\frac{m_i - m_{0i}}{f_i'}\right)\left[1 - \exp\left(-\frac{f_i'}{m_i}t\right)\right], \tag{2}$$

where v_0 is the velocity of the leukocyte cell at time $t = 0$ and z_i is the

height above the bottom. If N is the total leukocyte concentration in the liquid medium and P_i the relative partial concentration of each leukocyte type, we get for the number of leukocytes sedimented at the bottom

$$N_S = N \sum_i P_i z_i. \tag{3}$$

If z_i in relation (3) reaches the total height z_t of the liquid in the vessel, the sedimentation of the leukocytes of the i-type is completed.

The exposition of leukocytes from an immune individual to antigen might result in changes of the coherent vibration states as well as of the generated electromagnetic fields. For weaker fields the "rocking cloud" of attracted water dipoles is thinner that that for stronger fields (at the same frequency). The field with one frequency component may be connected with a thinner "cloud" than the field with several frequency components.

Figure 2 shows the computed curves of the leukocytes sedimentation. The thickness d_w of the attracted water molecules layer is assumed to be from 0 to 10 μm. The parameters of the leukocytes of various types used for the computation of the theoretical curves are given in Table 1. These parameters coincide with those commonly used. It follows from the computer results (not presented in this paper) that the sedimentation curves form depends on the f'_0 value as well as on the type pattern of the leukocyte population.

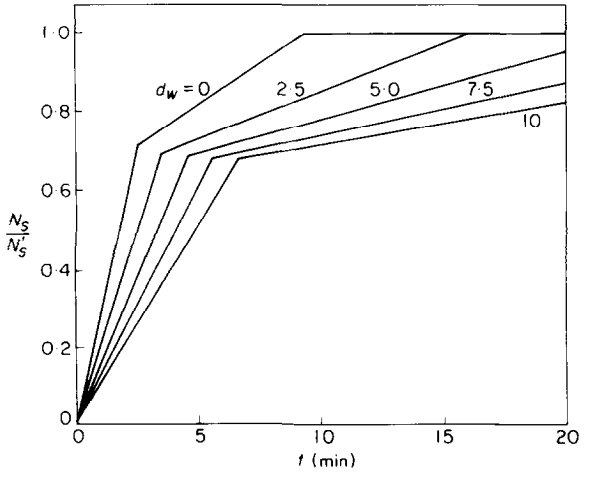


FIG. 2. Relative number N_S/N'_S of sedimented leukocytes against time (N'_S is the value of the sedimented leukocytes for $t \to \infty$). Computed theoretical curves. Thickness d_w (μm) of the attracted water dipole layer is a parameter. $v_0 \leq 0{\cdot}1$ m/sec, $z_t = 2{\cdot}35$ mm and $f'_0 = 5 \times 10^{-4}$ kg/msec.

TABLE 1

Parameters of leukocytes of various types used for computation of theoretical curves in Fig. 2. m_{is} is the relative specific mass

m_{is}	R_i (μm)	P_i
1·1	6·0	0·56
1·1	7·0	0·01
1·1	5·0	0·01
1·078	3·5	0·39
1·06	12·5	0·03

The preliminary experiments were carried out to observe the LAI test time course. The time course of the leukocyte sedimentation and adherence to the bottom surface of the vessel was recorded on a film. The preliminary experimental findings seem to support the theoretical prediction concerning the leukocyte sedimentation. The comprehensive experimental verification of the theoretical conjectures is under way and the results will be published in a separate paper.

4. Brownian Motion of Leukocytes at the Bottom

Let us neglect for the moment the proper active motion of the leukocytes. In such a case the Brownian statistics for a two-dimensional motion may be used to describe the leukocyte behaviour at the bottom of the vessel. The probability densities of the velocity v_i and of the position x_i of the free cells are given by

$$p(v_i) = \frac{m_i}{kT} v_i \exp\left(-\frac{m_i}{2kT} v_i^2\right),$$
$$p(x_i) = \frac{1}{2D^2 t} x_i \exp\left(-\frac{1}{4D^2 t} x_i^2\right), \tag{4}$$

where T is the temperature (in K), k is the Boltzmann constant, $D^2 = kT/f_i'$ is the diffusion coefficient and x_i is the distance along the plane bottom. Equation (4) may be easily derived from the relations given in (Papoulis, 1965). With the aid of equation (4) the probability density curves of the leukocyte velocity are computed and plotted in Fig. 3.

The Brownian motion for bounded particles (i.e. the motion in a potential well) is described by the probability density

$$p(x_i) = \frac{k_{0i}}{kT} x_i \exp\left(-\frac{k_{0i}}{2kT} x_i^2\right), \tag{5}$$

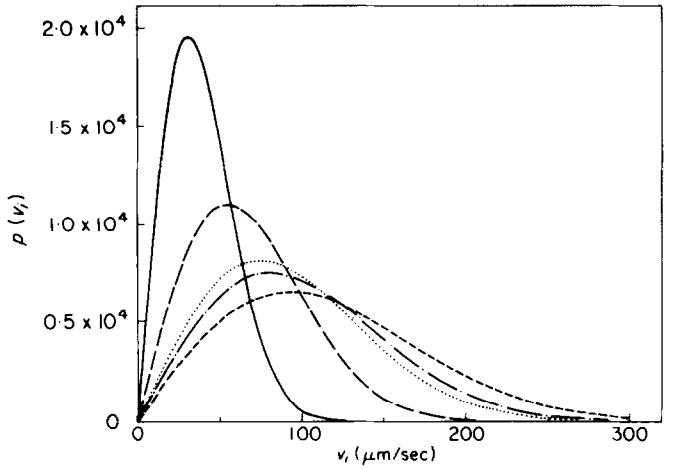


FIG. 3. Computed two-dimensional probability density of the velocity of free spherical particles (leukocytes) in a liquid medium. m_{is} and R_i are the relative specific mass and radius of the spherical particles. $T = 310$ K. Parameters are ——, $R_i = 10$ μm, $m_{is} = 1{\cdot}06$; — — —, $R_i = 6{\cdot}75$ μm, $m_{is} = 1{\cdot}1$; · · ·, $R_i = 5{\cdot}5$ μm, $m_{is} = 1{\cdot}1$; —·—·—, $R_i = 5{\cdot}25$ μm, $m_{is} = 1{\cdot}078$; – – –, $R_i = 4{\cdot}75$ μm, $m_{is} = 1{\cdot}1$.

where k_{0i} is the coefficient determining the force attracting the cell to a center. The computed probability density curves are plotted in Fig. 4. The higher the k_{0i} value the smaller the position fluctuations of the cells.

The magnitude of k_{0i} may depend on the electric field generated by the cell, on the electron density in the surface states of the bottom surface of the vessel and on the surface fluctuations of the electron density. The magnitude of the electron density is responsible for perpendicular attraction forces to the bottom. The fluctuations of the electron density (always present in any solid-state material) are responsible for tangential forces, i.e. these fluctuations create the potential wells for the cells.

The computed velocities of free cells in Fig. 3 seem to be too high in comparison with those observed visually but additional friction caused by the substrate surface is neglected in equations (4) and (5). Regardless of this the variance $2D^2t$ in the probability density of the leukocytes' position in equation (4) depends on time and, therefore, the leukocytes should be carried away from their initial positions. On the contrary the motion of the majority of the leukocytes is similar to the oscillatory one around a fixed center without any drift. This follows from the visual observation and evaluation of the film records of the preliminary LAI test experiments. The total population of the leukocytes at the bottom consists of leukocytes that adhere to the bottom, of leukocytes oscillating around a center and

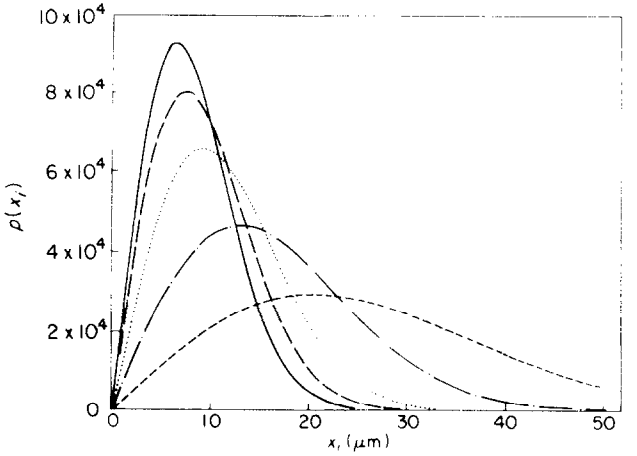


FIG. 4. Computed two-dimensional probability density of the position of spherical particles (leukocytes) for the bounded Brownian motion in a potential well. $T = 310$ K. Values of k_{ai} (kg/sec^2) are ——, $1{\cdot}1 \times 10^{-10}$; — — —, $0{\cdot}75 \times 10^{-10}$; · · ·, $0{\cdot}5 \times 10^{-10}$; — · — · —, $0{\cdot}25 \times 10^{-10}$; – – –, $0{\cdot}1 \times 10^{-10}$.

of free moving leukocytes, but only a small fraction of the leukocytes exhibits a free motion. These cells ruffle.

If the probability densities similar to those in Fig. 4 were experimentally specified, the k_{0i} coefficients would be found. In this way the interaction forces between the leukocytes and the surface of the vessel might be specified. We present only a rough approximate assessment of the interaction energy. A spherical particle with a diameter 10 μm, relative specific mass 1·1 and velocity 100 μm/sec has the kinetic energy of the order of 10^{-21} J. For the electric field intensity 10^5 V/m, the surface charge density 10^{19} electrons/m^2, the surface charge change (removal) in the circle of the radius 0·1 μm we get for the work on the path of 0·1 μm the value of the order of 10^{-16} J. This represents the force (of the order of 10^{-9} N) in the direction perpendicular to the surface. If the relative fluctuations of the surface charge density are of the order of 1%, then the energy representing tangential forces may be of the order of 10^{-19} J, i.e. two orders greater than the kinetic energy given above (the energy of the Brownian motion).

5. Time Dependence of Leukocyte Adherence to the Solid-state Surfaces

For the time dependence of the leukocyte adherence the following equation may be used

$$\frac{dN_a}{dt} = A(N_S - N_0 - N_a)F, \tag{6}$$

where N_a, N_0 are the numbers of the leukocytes that adhere to the bottom and of those that do not adhere at all, respectively, A is a constant and $F = F(k_{0i}, x_i, x_N)$ is the adherence probability density (x_N representing the dimension of the surface charge density fluctuations). It follows from equation (6) that

$$L = \frac{N'_S - N_a}{N'_S} = 1 - \frac{N'_S - N_0}{N'_S}[1 - \exp(-AFt)], \tag{7}$$

where N'_S is the value of N_S for $t \to \infty$.

Equation (7) is derived under the assumption that both N_S and N_0 do not depend on time, but at least the time dependence of N_0 should not be neglected. The N_0 value depends on the antigen action. We make a tentative assumption that

$$N_0 = N'_0\left[1 - \exp\left(-\frac{t}{\tau}\right)\right], \tag{8}$$

where τ is the time constant of the antigen action. If we neglect the antigen action ($\tau \to \infty$ in equation (8)), the time dependence of the LAI test index L is given by a simple exponential curve given by equation (7). If the antigen action is assumed and equation (8) is used, the curves $L = L(t)$ computed from equation (7) have the form given in Fig. 5. The main

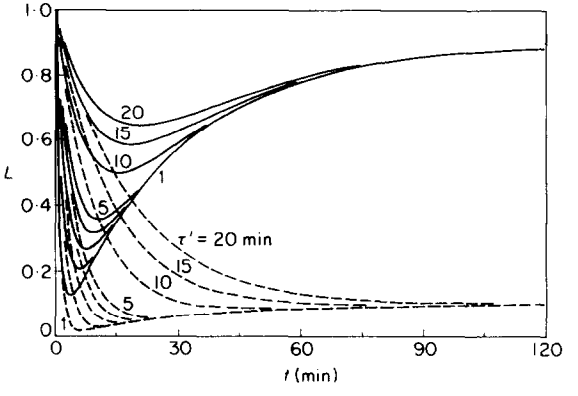


FIG. 5. Computed LAI test index L against time. $N'_0 = 0{\cdot}9$ (full lines) and $0{\cdot}1$ (dashed lines). $\tau = 30$ min and $\tau' = 1/AF$ is the parameter of the curves.

difference between the two families of curves in Fig. 5 is rooted in the number N_0 of the leukocytes that do not adhere at all, as follows from equations (7) and (8). The antigen action probably changes the vibration

mode pattern and consequently even the generated electromagnetic field of the majority of the leukocytes from an immune individual. As a result of this antigen action the attractive forces exerted by the cell-generated field are weak in comparison with the Brownian motion forces. This may be significant for leukocyte adherence inhibition even if the final mechanism of adherence is quite different in nature. Observation of the adherence process is described elsewhere (e.g. Witkowski & Brighton, 1972; Grinnell, 1978).

6. Conclusion

The theoretical analysis presented in this paper is based on the assumption that a high frequency electromagnetic field generated by Fröhlich coherent vibrations in living cells may have appreciable values within a distance of about 10 μm outside the cellular surface. This electromagnetic field may undergo significant changes during the cell cycle and may depend on biological conditions.

The electromagnetic field of leukocytes dispersed in a liquid medium acts on the dipolar molecules, causes their orientation (rotation and rocking) and attracts them to the outer surface of the membrane. In this way a "liquid rocking cloud" is formed. This "cloud" moves together with the cell. For the higher values of the electromagnetic field intensity the "rocking cloud" is thicker and the friction resistance withstanding the cellular motion is greater than for the lower values of the intensity. This may cause the differences of the leukocyte sedimentation in the initial time of the LAI test. If leukocytes from an immune individual generate weaker fields than do those from a non-immune one, then the former should have a considerably higher sedimentation rate than the latter. But the whole vibration mode pattern should be considered with the vibration frequency and energy changes and with the multimode–single mode and multipole–dipole oscillation transitions.

The cell-generated electromagnetic field interacts with the mobile surface charge of solid-state materials. This interaction results in the attractive forces between the leukocyte and the surface. Surface charge density fluctuations create the potential wells for the leukocytes. Some of the leukocytes, at the flat bottom of the vessel, should oscillate around a fixed center as if they were trapped in such potential wells. The antigen action most likely changes the coherent vibrations and the field generated by the leukocytes. The decrease of the electric field intensity results in the decrease of attractive forces to the solid-state surfaces and leukocyte adherence inhibition is reached.

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