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About the Method of Increasing the Effectiveness of Antibiotics Using Microwaves

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Abstract

The formula for the natural frequency of torsional vibrations of the DNA helix is obtained by methods of mathematical physics. The data on the dependence of *E. coli* survival on the time of exposure to centimeter waves were analyzed, the numerical coefficient in the resulting formula was determined. According to this formula, the resonant frequency for the DNA of another strain of *E. coli* was found. When irradiated with centimeter waves of this frequency, a significant decrease in the survival rate of another strain of *E. coli* was obtained. Similar experiments on three other bacterial cultures confirmed the validity of the formula obtained.

Subject Areas

Pharmacology

Keywords

Microwaves, DNA, Torsional Vibrations, Resonance, Survival

1. Introduction

In 95% of cases of infectious cystitis, its causative agent is *E. coli*. To suppress it, various antibiotics are used, kanaferon (Germany), cystone (India), "5-NOK" (Russia), etc. However, cystitis can turn into a chronic form, or the bacteria mutate, acquiring immunity to antibiotics, or form drug-resistant bacterial films. The result of the transition to a chronic form in women can be infertility.

The problem of addiction of pathogenic bacteria to antibiotics is of a general nature. Therefore, the task of creating such a treatment system that would exclude the habituation of bacteria to drugs is urgent. One of the ways to solve this problem is the use of centimeter electromagnetic radiation.

Extensive literature is devoted to the action of electromagnetic waves, including the visible range, UV, IR, extremely high frequency (UHF) and the terahertz range.

The bactericidal effect of centimeter waves (ultrahigh frequency, microwave) on media with *E. coli* was discovered back in 1958 [1].

The ecological aspect of the microwave effect on bacteria is considered in [2].

In [3], the authors investigated the effect of an electromagnetic field (EMF) with a frequency of 136 Hz on the *E. coli* B strain and concluded that EMF of this frequency inhibits the division of *E. coli* cells without causing their death.

It was shown in [4] that the effect of microwave EMF of the order of several tens of gigahertz on short DNA of lymphocytes leads to a sharp increase in the number of single-strand DNA breaks, the mechanism of action is not disclosed.

In [5] it is assumed that "the molecular structure of genetic information carriers is a spiral resonator or a chain of a large number of strongly coupled oscillatory circuits. As a result of exposure to a high-frequency field, genetic information cannot be read, which leads to the loss of the reproductive function of a biological object."

Indeed, in DNA, as in a solenoid, a parasitic resonance can occur, because in DNA there is a system of generalized-connections. However, the calculation shows that this assumption is incorrect. The frequency of free oscillations of the circuit is $f = 1/2\pi\sqrt{LC}$. The inductance of the solenoid $L = \mu_0 \mu sN/l$, μ_0 is the magnetic constant, μ is the relative magnetic permeability of the core material (depends on frequency), s is the cross-sectional area of the core, l is the length of the midline of the core, N is the number of turns. Magnetic constant $\mu_0 \approx 10^{-6}$ Gn/m. Since we need to estimate only the order of magnitude, we can assume, without using tables, that the intrinsic capacity of a single-layer coil in the form of DNA in pF is numerically approximately equal to the radius of the "winding" in cm.

The diameter of the DNA coil is 2 nm, the parasitic capacity is 10^{-7} cm, picofarad = 10^{-19} farad. The length of *Escherichia coli* DNA is approximately 10^{-3} m. The area of the coil = 3.14×10^{-38}. The step size of the spiral is 3.4×10^{-9} m. Number of turns = length/step size. Then the parasitic resonance of DNA is $f \sim (0.584 \ldots 29.20) \times 10^{24}$ Hz, which significantly exceeds the frequency of gamma radiation of 10^{19} Hz, therefore, there is no parasitic resonance of DNA at frequencies of the order of 10 GHz.

As a mechanism of adaptation of *E. coli* cells to non-thermal extra high frequency EMF in [6], where *E. coli* cells were exposed to EMF from 8.5 GHz to 18 GHz at an exposure of 5 - 110 minutes, the effect of microwave EMF on the expression of specific genes of *E. coli* DNA was proposed.

This work is devoted to the study of the mechanism of the effect of centimeter waves on DNA.

2. Theoretical Definition of Resonance

It is known that excited DNA emits microwave electromagnetic waves associated with torsional vibrations of its spiral [7]. DNA excited by a picosecond laser pulse emitted waves of a certain frequency, the magnitude of which the authors estimated at several gigahertz.

Therefore, DNA must also absorb electromagnetic waves in this range.

Since the DNA helix has its own frequency of torsional vibrations, an external effect on the DNA of an EMF with a frequency equal to the natural frequency of vibrations of its spiral will cause a resonance that interferes with the preparation of the cell for mitosis and leads to its death.

The ring DNA of *E. coli* is superspiralized (superspiralized), which is typical for ring DNA. Before DNA replication, the cell unfolds a superspiral, including in order to "check" the integrity of the macromolecule, whether anything unnecessary has stuck to it, etc. If the DNA is damaged, the cell "postpones" division and heals the DNA. If the microwave EMF begins to act, the DNA begins to fold back into the superspiral. Thus, the microwave interferes with the breeding of the superspiral, replication is delayed, and the cell postpones division until better times. It is impossible to postpone division indefinitely, because the cell dies after a certain characteristic time exceeding the time of the cell cycle.

Macromolecules of nucleic acids are natural polyampholytes. Pairs of purine and pyrimidine bases adenine-thymine (A-T) and guanine-cytosine (G-C) forming the DNA polynucleotide chain, as asymmetric formations, are dipoles. Thus, the DNA helix is a system of multidirectional dipoles.

The energy of the electromagnetic wave $E = h\nu$, where h is Planck's constant, ν is the frequency in hertz. For microwave radiation at $\nu = 1$ - 20 GHz, this energy is (0.7 - 13) 10^{-24} J or (0.4 - 8) 10^{-5} eV, which is 3 orders of magnitude less than the thermal energy $kT = 0.03$ eV (k—Boltzmann constant, $T = 300$ K − room temperature), 4 orders of magnitude less than the hydrogen bond energy of 0.1 eV, 5 orders of magnitude less than the covalent bond energy (1 - 10) eV. However, the microwave energy is sufficient to excite the rotational levels of the basic electron-vibrational state of some molecules, clusters of water molecules also resonate under the influence of microwave, on which the action of microwave ovens is based.

The DNA molecule consists of billions of atoms, so the effect of microwave radiation on DNA is not quantum, but classical. The "protein-machine" hypothesis suggests that EMF leads to the excitation of vibrations of a macromolecule as a solid body [8].

In the classical approximation, the effect of EMF on DNA is described by the Umov-Poynting vector S—the vector of density of the EMF energy flow through a unit area normal to S, per unit time (otherwise, the power flux density):

$$\boldsymbol{S} = [\boldsymbol{E}, \boldsymbol{H}]$$

where E and H are the electric and magnetic field strength vectors, respectively.

In [7], the emitted wave is caused by vibrations of a system of elementary dipoles (Hertz oscillators) located along the length of *Escherichia coli* DNA. The same system of dipoles interacts with the electric field E in the vector S of incident wave.

The non-thermal power flux densities used—of the order of 0.1 mW/cm^2—are quite sufficient to excite torsional vibrations of the DNA helix.

Torsional vibrations of DNA were modeled in [9], where for 400 pairs of nucleotides it was shown that frequencies in the region of 1 THz are present in the spectrum of vibrations of the molecule, they correspond to acoustic torsional vibrations of the DNA chain.

The formulas for the natural frequency of torsional vibrations of a curved spiral are extremely complex, the frequency is not expressed explicitly. In addition, the rigidity of the DNA helix is unknown, which depends on the compaction of DNA.

For simplification, the model of DNA as an elastic single rod is used. The fact that DNA behaves exactly like a rod during twisting is shown in [10] [11].

Instead of the Schrodinger equation, to get an expression for the natural frequency, you can use the Lagrangian formalism, from the Lagrange equations

$$\frac{\mathrm{d}}{\mathrm{d}t}\frac{\partial L}{\partial \dot{q}_i}-\frac{\partial L}{\partial q_i}=0,\ i=1,2,\cdots,n, \tag{1}$$

where L is the Lagrange function of a system with n degrees of freedom is defined in terms of kinetic energy T and potential energy U as follows:

$$L(q,\dot{q})=T(q,\dot{q})-U(q). \tag{2}$$

Here $q,\dot{q}$ are the vectors of generalized coordinates and generalized velocities, respectively.

Consider a rod with two twisting discs at the ends. This model corresponds to the twisting of the DNA helix. We denote the torsional rigidity of the rod by G; the moments of inertia of the disks relative to the longitudinal axis of the system by J_1 and J_2. As generalized coordinates, we take the angles of rotation of the disks φ_1 and φ_2 relative to some initial position in which the rod is not twisted. The Lagrange function (2) for the model under consideration will take the form

$$L=\frac{J_1\dot{\varphi}_1^2}{2}+\frac{J_2\dot{\varphi}_2^2}{2}-\frac{G\cdot(\varphi_2-\varphi_1)^2}{2} \tag{3}$$

Substituting (3) into (1), we obtain a system of differential equations:

$$\begin{aligned} J_1\ddot{\varphi}_1-G\cdot(\varphi_2-\varphi_1)&=0,\\ J_2\ddot{\varphi}_2+G\cdot(\varphi_2-\varphi_1)&=0. \end{aligned} \tag{4}$$

The solution of system (4) is sought in the form

$$\varphi_j=A_j\cdot\mathrm{e}^{i\omega t},\ j=1,2. \tag{5}$$

Substituting (5) into Equations (4) leads to a homogeneous system of algebraic equations for determining coefficients A_p, which has solutions only for certain values of the frequency ω. The latter must be determined from the condition

$$J_1 J_2 \omega^4 - \left(J_1 G + J_2 G\right)\omega^2 = 0. \quad (6)$$

The biquadrate Equation (6) has solutions:

$$\omega_1 = 0, \omega_2 = \sqrt{G\frac{J_1 + J_2}{J_1 J_2}}. \quad (7)$$

The zero frequency, due to the degeneracy of the system, corresponds to a solution that describes the uniform rotation of the entire system as a rigid whole (without twisting the rod). The second frequency value in (7) is the desired natural frequency. Assuming the molecule is symmetric, *i.e.* assuming $J_1 = J_2 = J$ finally we get for the linear frequency $f = \frac{\omega}{2\pi}$ an expression similar to the expression for the frequency of torsional oscillations of a spring pendulum

$$f = \frac{1}{2\pi}\sqrt{\frac{2G}{J}} \quad (8)$$

where J is the moment of inertia of the DNA helix relative to the axis passing through the centers of its turns. From formula (8) follows an extremely important conclusion for subsequent reasoning. The fact is that the value of J is proportional to the length of the molecule, *i.e.* proportional to the number of nucleotide pairs of N. Thus, the resonant frequency of the EMF should be inversely proportional to the square root of N:

$$f = kN^{-1/2} \quad (9)$$

where the coefficient k is determined empirically. This is the general formula of torsional vibrations of any DNA. The coefficient k integrally includes the compactification of the DNA helix.

Bacterial DNA is not linear, they are closed. However, formula (9) remains valid in view of the ratio $d \ll L$, where d is the diameter of the DNA helix, L is its length.

Note that the equations do not take into account the DNA environment, effective friction, which should lead to a decrease in the frequency of the driving force.

3. Experimental Determination of Resonance

In [12], the effect of microwave in the range from 8.82 GHz to 10.4 GHz at a power flux density of 0.2 mW/cm^2 on cells of the mutant strain of *E. coli hcr* "*exr*" was studied, a significant decrease in the survival of *E. coli* hcr'exr' by more than 50% was obtained.

We recalculate the dependence of *E. coli* survival on the time of exposure to EMF in [12] on the dependence of survival on frequency.

The graphs in **Figure 1** show that when a certain exposure time (120 min.) is reached, the curve acquires a distinct resonant appearance. The resonance point

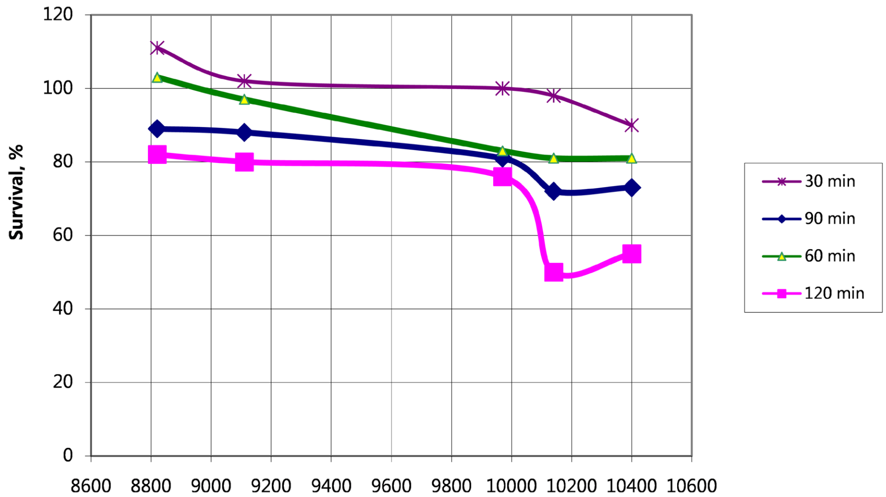

Figure 1. Frequency, MHz.

is 10.14 GHz, at this frequency the maximum reduction in survival is achieved.

Knowing this frequency and length of DNA *E. coli hcr*⁺*exr*⁺, it is easy to get the coefficient $k = 21.75$. Thus, formula (9) is completely defined.

To test the method, the *E. coli* ATCC 25922 was exposed to microwave EMF irradiation. DNA of *E. coli* ATCC 25992 DNA contains 5,130,767 nucleotide pairs. Then, according to the formula (9), the natural frequency of torsional vibrations of its spiral is 9.6 GHz.

In the experiment, a daily broth culture was used, tenfold dilutions of the culture in isotonic sodium chloride solution were prepared: working dilution 1:100 (10^{-2})—control and experimental samples. Culture dilutions exposed to microwave exposure (control and experimental samples) were used to prepare consecutive tenfold dilutions up to 10^{-7}. Lawn sowing was carried out on a dense nutrient medium (nutrient agar) 0.1 ml of dilutions 10^{-6} and 10^{-7} of control and experimental samples in two repeats. The cups were placed in a thermostat for 24 hours at a temperature of 37˚C. The results were recorded by counting colonies in cups.

The source of the microwave radiation was an Agilent Technologies E82570 1 generator, on a Gann diode that creates harmonic polarized oscillations (a 1-watt H10 wave), the direction of the electric field vector is located in the vertical plane.

The power flow density is non-thermal, no higher than 2.5 mW/cm^2 with losses of no more than 20%. Exposure time—120 min.

The test tubes were insulated with black paper with the absence of heavy metals in the black paint, so as not to shield the microwave EMF. The ambient temperature was controlled by a thermometer and changed during each experiment by no more than 0.5˚C. On different days of the experiments, the temperature ranged from 24˚C to 27˚C.

Dependence of *E. coli* survival on heating

$$K = \left(N_0 - N_k\right) / N_k \Delta T$$

where N_0 is the number of viable cells or infectious units in culture when heated at 1˚C for 60 minutes, N_k is the initial number of viable cells or infectious units in culture at the initial temperature, ΔT is the temperature change when the suspension is heated. However, in our stationary case and taking into account equal conditions for control and experimental tubes, temperature changes can be neglected.

In the experiments described above with irradiation of *E. coli* cultures the following results were obtained: at frequencies of 9.2 GHz, 9.4 GHz, 9.8 GHz, 10 GHz, no significant changes in survival were observed, at the resonant frequency of 9.6 GHz, survival decreased to 22.5%.

A similar experiment was conducted with an *E. coli* M17 culture with a DNA length of 4,483,110 bp. At frequencies of 9.8 GHz, 10 GHz, 10.4 GHz, no changes in survival were found in comparison with the control, at the estimated frequency of 10.263 GHz, survival decreased to 28.7%.

A similar experiment was conducted with cultures of *M. avium* 104 and *Mycobacterium tuberculosis* H37Rv (Pasteur) ATCC 25618.

Cultures of *M. avium* and *Mycobacterium tuberculosis* were rubbed with glass beads in a nutrient liquid medium Middlbrook 7H9 using Vortex. Defended 10 min. They were transferred to a sterile test tube and adjusted with a nutrient liquid medium Middlbrook 7H9 according to the turbidity standard No. 5 of the GNISC (5×10^8 CFU/ml). Bred 10 times. Glass tubes with a diameter of 15.5 mm and a wall thickness of 1 mm were carefully closed with sterile rubber stoppers. The height of the column of liquid with microorganisms is 10 cm.

At frequencies of 9.8 GHz, 10 GHz, 10.5 GHz, no changes in *M. avium* survival were found in comparison with the control, at the estimated frequency of 10.31 GHz, a decrease in survival was observed to almost zero.

At the frequencies of 9.8 GHz, 10.1 GHz, 10.6 GHz, no change in the survival rate of *Mycobacterium tuberculosis* was detected, at the estimated frequency of 10.36 GHz, a decrease in survival rate to 0.1% was observed.

A direct experiment of microwave EMF absorption in *E. coli* M17 culture showed that the maximum absorption is observed at a frequency of 10.27 GHz, which is very close to the frequency calculated by formula (9) of 10.263 GHz [13].

Note that the fact that effective frequencies were calculated without friction, that indicates that this friction is extremely small.

4. DNA Resonance at Higher Frequencies (Harmonics)

In [14], the effect of NT MWs on the repair of radiation-induced DNA breaks in *E. coli* K12 AB1157 was studied by the method of dependence of abnormal viscosity on time. Significant inhibition of DNA repair was found when X-ray irradiated cells were exposed to MWs in the frequency ranges 51.62 - 51.84 GHz and 41.25 - 41.50 GHz. The effect had a pronounced resonant character with resonant frequencies of 51.755 GHz and 41.32 GHz, respectively.

According to their formulas (9), it is obvious that these frequencies cannot be the natural frequencies of torsional vibrations of the DNA helix of *E. coli*, moreover, there cannot be an additional close resonance.

The length of *E. coli* K12 AB1157 is 511,174 Mb, respectively, the natural frequency of torsional vibrations of the DNA helix is 9.620 GHz.

But the frequency of the driving force can be an overtone, i.e. in *n* times greater than the natural frequency where *n* is an integer.

If the DNA chain is torn in half during X-ray irradiation, then half the length and half the number of nucleotide pairs. According to formula (1), the natural frequency of the DNA halves is 13.6047 GHz. With a good degree of accuracy 41.32:13.6047 = 3. Additional resonance is obtained due to the fact that the circuit is not torn into equal parts.

From this it can be concluded that in addition to hindering replication and single-strand breaks, there is another mechanism for reducing bacterial survival by microwaves—inhibition of DNA self-repair due to the excitation of DNA torsional vibrations. It is this mechanism that plays a major role in those experiments in which bacterial cultures are in saline, that is, an increase in the number of single-strand breaks is unlikely due to the fact that DNA is protected by the bacterial shell, and replication is practically absent.

5. Conclusions

We found out that EMF in the centimeter range, the frequency of which coincides with the natural frequency of torsional vibrations of DNA spirals of pathogenic bacteria, 1) prevents DNA replication, which leads to the death of bacteria, 2) dramatically increases the number of single-strand breaks in the DNA chain, 3) suppresses DNA repair systems.

The method can be used to suppress the reproduction of any cells, both pathogenic bacteria and malignant neoplasm cells. Can a microwave EMF reach a lesion, for example, tuberculosis or a tumor?

The depth of the skin effect for copper at 1 GHz is 0.2 microns. The depth is inversely proportional to the root of the conductivity. The conductivity of water is 12 orders of magnitude lower than that of copper, *i.e.* the thickness of the skin layer is 6 orders of magnitude greater. That is, 103 millimeters = 1 meter. For seawater, the thickness of the skin layer is less than 2 cm, but the salinity of seawater is 35%, and blood is 1%. In addition, at frequencies of the order of 0.1 kHz, blood behaves like a conductor, at microwave—like a dielectric.

In addition to the allocated resonant frequency of 2.4 GHz (resonance occurs on clusters, water passes other frequencies in the microwave range.

In the medical literature, it is noted that the depth of penetration of the microwave into the human body is 8 - 12 cm. But only for therapeutic warming up in phthisiology, which dramatically reduces the thickness of the skin layer, because it makes the tissues more conductive. Non-thermal microwave radiation passes freely through the human body.

Since microwave EMF significantly reduces the survival rate of pathogenic bacteria, it can help increase the effectiveness of antibiotics that are used against these bacteria.

At the same time, the habituation of bacteria to antibiotics does not occur, because microwave EMF suppresses the adaptation system of bacteria.

Conflicts of Interest

The authors declare no conflicts of interest.

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