METHODS

Feasibility of Novel Atomic-Molecular Resuscitation Tools

L. A. Piruzyan

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Complex multifactor diseases are characterized by enhanced formation of toxic free radicals. The developed apparatuses based on electron paramagnetic resonance or nuclear magnetic resonance electrodialysis are therapeutic tools playing the role of atomic-molecular "artificial kidney" excreting anion and cation radicals from the organism. These tools can be used in medicine in combination with drug therapy to protect cells from toxic action of free radicals produced during metabolic neutralizing deactivation of exogenous toxic substances invading the organism.

Key Words: *medical biophysics; magnetic field; spin effects; membrane phospholipids; electron paramagnetic resonance electrodialysis; nuclear resonance electrodialysis*

The permanent lack of efficient therapeutic procedures can be explained by poor study of the pathogenic mechanisms of various diseases. The fundamental sciences make untiring efforts to develop novel approaches to the therapy of various pathological states [1-4]. Among them, of particular interest is application of such diagnostic tools as nuclear magnetic resonance (NMR) and electron paramagnetic resonance (EPR), which are widely spread in practical medicine. Studying the processes developing during the work of these sophisticated medical tools and the effects of the concurrent electromagnetic radiation under normal and pathological conditions can help developing novel methods to prevent and treat numerous diseases.

The search for the new methods to control the performance of biological organisms based on novel data harvested in physics, the effects of electromagnetic fields included are now in progress. The development of perspective resuscitation equipment working at the atomic-molecular level makes it possible to perform resuscitation procedures at the initial state of fading organism. In case of complex critical states, such equipment can be used in anesthesiology, resuscitation, and intensive care medicine. The atomic-molecular resuscitation equipment can be efficiently coupled with defibrillation procedures employing energy generated by EPR and NMR (Zeeman effect). Combined effect of defibrillation, electron paramagnetic resonance electrodialysis (EPRED), and nuclear magnetic resonance electrodialysis (NMRED) on living organism needs further studies, since a powerful short-term (0.01 sec) electric impulse can produce a positive effect in stimulation of vital activity at the atomic-molecular level followed by engagement of Zeeman effect. (Pieter Zeeman, [1865-1943],a Dutch physicist known for his works on optics, magnetooptics, and atomic spectroscopy. In 1896, he discovered splitting of energy levels of atoms and molecules in magnetic field [MF] referred thereafter as Zeeman effect). Placed into external MF, an atomic system

N. N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, Moscow, Russia. ***Address for correspondence:*** rla2001@mail.ru. L. A. Piruzyan

with intrinsic magnetic moment acquires an extra energy, which can be viewed as atomic-molecular energy stimulation. In living organism, a persistently developing pathology is characteristic of late stages of vital activity. As a rule, there is little chance to restore the most important physiological functions degraded at these stages.

In 1980s, a complex setup with a magnetic chamber was constructed in N. N. Semenov Institute of Chemical Physics to examine human patients and biological objects under the effect of energy produced by microwave electromagnetic field aimed to directly and selectively control the biological physicochemical reactions. The magnetic chamber was designed in Central Research Institute (Ministry of Medium Machine Engineering) and in D. V. Efremov Research Institute of Electrophysics Equipment. The aim was to create an apparatus for high-resolution nuclear magnetic tomography for *in vivo* detection of biochemical processes in organs and tissues. It is noteworthy that generation of potent magnetic fields is a rather difficult technical problem, which was successfully solved in a SP-172 apparatus. This large-scale electrotechnical apparatus generated MF with magnetic intensity 10 Oe in the volume ranging from 0.7 to 2.5 m^3. The mapping measurements established that special homogeneity of MF in 1 m^3 was no less than 10^{-6}. Thus, the most challenging technical features in the development of nuclear magnetic tomography for noninvasive examination of large-scale biological objects had been solved in 1983. The microwave irradiation was carried out simultaneously with application of constant magnetic field (CMF) during EPR or NMR examination under persistent monitoring the dynamics of physiological functions of the organism with possibility to use the parenteral dialysis [2].

The reliable statistical data on the effect of CMF at different structural levels of biological objects were obtained by the early 1980s. It has been established that CMF reduced the concentration of free radicals (FR) in organs and tissues with tits normalization in 2-3 weeks. The character and dynamics of these changes correlated with changes in *1*) activity of cytochrome C oxidase in hepatic and renal mitochondria, *2*) electron microscopic and optical histological images, *3*) erythropoietic functions, and *4*) affinity of hemoglobin to oxygen. Additionally, it had been established that moderate CMF provoked no appreciable alterations in cellular, subcellular, tissue, and the systemic organization of living organisms.

Therefore, the data confirmed our hypothesis on the role of chemical agents with unpaired electron (FR included) in the realization of CMF effects at various structural levels of biological objects. This hypothesis was also corroborated in studies demonstrating that the energy of interaction of the molecules with an unpaired electron under CMF of about 0.1 T is comparable with the energy of spin-spin interaction at the intermolecular distances [2]. The concepts of spin chemistry contributed to the armory of modern science. Outstanding contributions to the development of this scientific field were made by Academicians Ya. B. Zeldovich, A. L. Buchachenko, and PhD E. L. Frankevich.

The frontier studies on the eve of technological breakthrough

The spin and magnetic effects in biological systems: exclusive role of membrane phospholipids. With clear appreciating the complexity of reviewing modern theoretical and experimental data dealing with the action of MF on biological objects, we focus only on MF effects related to membrane phospholipids [5]. Here we consider the phenomena caused by the effects of electron and nuclear spins on the rate of FR in model biological media and native tissues, and discuss experimental data and theoretical inferences on the effects producing by MF exerts on LPO processes in biological membranes with involvement of FR and triplet molecules.

The theory of MF effects on LPO in membranes is based on the mechanism of MF action on recombination rate of the radical pairs of membrane phospholipids due to induction of singlet-triplet (S-T) transitions during lifetime of the radicals. Magnetic interaction involves the elementary particles (electrons, proton, *etc*.), the electric currents, magnetized bodies that possess magnetic moment originating from spin of the elementary particles.

In oxygen-containing medium, the oxygen molecule rapidly binds to one of carbogen atoms with unpaired electron (usually to the most remote atom in a molecule) producing a lipid peroxide radical. It is a common knowledge that oxidation of unsaturated fatty acids comprises several stages of FR production before their elimination. Combination of various types of FR produced during oxidation of the lipids at different stages of chemical reactions assumes the presence of some MF-sensitive stages both in FR reactions and in interaction between iron atoms with oxygen. Since some lipids located in the membrane structures are involved in metabolic, effector, regulation, and structural functions, the effect of MF on recombination of the radical pairs in the membranes can be especially pronounced.

Actuality of the studies aimed to reveal the effects exerted by MF on LPO processes and considerable promise of the currently implemented research work is now evident from biological and biophysical vistas. Due to spectacular works of Academician A. L. Bu-

chachenko, the spin chemistry is now considered as a separate and promising branch of quantum chemistry.

On involvement of FR in generation of membrane potentials. The study of biopotential generation mechanisms can be instructive to reveal their relation to FR. Under certain assumptions, it is possible to establish theoretically the dependence between membrane conductance and FR concentration. Interrelation between FR and biopotentials is closely related to transfer of energy and electric charge in the biological systems. Numerous examinations of EPR spectra of biological objects showed that all actively metabolizing systems (frozen or lyophilized in active state) contain unpaired electrons in concentration of 10^{10}-10^{17} spin/g dry weight or 10^{9}-10^{10} spin/g wet weight [5-7]. Generation of biopotentials results not only from concentration gradient of certain ions, but it can originate from the free radical states and semiconductivity phenomena in biological structures.

Effect of CMF on FR concentration in mouse organs and tissues

The effect of CMF on electrical properties of neuron axons, on spatial orientation of biological molecules, and on processes related to magnetic phenomena involving water and electrolytes in living organisms is a promising avenue of investigation. Deciphering of the primary mechanism of CMF effects in many biological events needs further experimental and theoretical efforts [6,7].

Changes in K^+, Na^+, and Ca^{2+} concentrations in ascitic fluid of tumor-bearing animals induced by CMF. Exposure of an animal to CMF with magnetic intensity of 5×10^3 Oe for 3-5 days significantly increases concentration of K^+ ions in extracellular ascitic fluid. At the same time, concentrations of Na^+ and Ca^{2+} ions do not change significantly [19].

Effect of CMF on sarcoma 37 ascitic tumor. In experimental animals, CMF delayed tumor progression. Moreover, it moderated accumulation of ascitic fluid, the growth of tumor cells, and the changes in concentration of free radical states. The linear character of dependence of inhibitory effects (according to specified criteria) on exposure time suggests that MF can directly affect the mechanisms underlying the development of ascitic tumor [10].

Some studies focused on the content of electrolytes in the extracellular ascitic fluid of tumor-bearing animals. They measured concentrations of K^+, Na^+, and Ca^{2+} in the experiments with different exposure time and the periods of CMF application during tumor progression. These concentrations were also measured in protein solutions at various terms after termination of CMF exposure [11].

The role of biochemical processes in responses of biological systems to MF. Theoretical consideration of the MF effect on the rate and directivity of chemical reactions can be fundamental in explanation of the effect of MF on a number of biochemical reactions. This consideration is based on the theory of chemical polarization of nuclei and electrons. Versatility of chemical reactions that demonstrate the chemical polarization of the nuclei indicates this phenomenon as universal process in biochemical and biophysical fields. The primary target of MF in a living system can be the biochemical reactions. The integral biomagnetic effect in total organism caused by modification of biochemical reactions at some stage of the metabolic system results probably from transformation of the molecular processes effected across the adaptive and protective barriers at the cellular, regional, or organism levels. Analysis of biomagnetic effects can efficiently employ the regularities of interaction between the molecular and systemic levels of coordinated performance of biological systems [18].

Geomagnetic stochastic control over life activity

The universal timing signal needed for living cells is provided by daily geomagnetic variation of solar radiation denoted by Sq (Solar quiet). Sq-variation results from axial rotation of the planet with the period of 24 h. For the discussed MF-produced biological effects, this variation plays the role of external force or "pumping". The biological rhythm is a specific example of oscillatory process, which obeys to certain universal requirements of general theory of oscillations. Specifically, an oscillatory system tuned to the amplitude resonance induced by an external force must have the natural frequency greater than the pumping frequency; the differences in these frequencies is "used" by the system to compensate friction (resistance) which otherwise would damp the oscillations [12,13].

In the University of Pittsburgh (USA), the magnetic resonance imaging was employed to simultaneously determine O_2 blood level in every cubic millimeter of various brain subdivisions during complex mental process and operation scheduling performed as a part of complex study of the cerebral work.

On February 28, 1998 the researchers from the Johns Hopkins University, demonstrated novel software for functional magnetic resonance tomography, which in the following was widely used to examine cerebral activity. This method can assess the amount of blood passing through a specific cerebral region. The experiments on rats carried out in Prof. P. C. van Zijl lab demonstrated that the new method made it possible not only to assess the arterial inflow, but also to measure the amount of oxygen utilized in the particular region.

The novel method employed the difference between the magnetic properties of two fractions of hemoglobin molecules, with and without oxygen. The novel technique can be used not only to accurately measure activity in various brain subdivisions during execution of different tasks, but also to prognosticate the development of disturbances in cerebral circulation [15].

On hypothetical material nature of thought

A principal problem in physiology and medicine is the search for material correlates of intellectual activity (thinking) and for the biochemical and biophysical reactions in the brain that initiate and implement it [9].

Electroencephalogram is an integral presentation of ionic conductance variations in the membranes of huge number of neurons generating the postsynaptic potentials, which is characterized by high spatial and especially temporal resolution. The extra- and intracellular processes reflected in EEG should be accompanied by the changes in physicochemical indices of the blood circulating in the brain and perfusing its specific subdivisions. What are the molecular processes that initiate these events? Recording of cerebral arteriovenous FR difference during acquisition of object recognition (or non-recognition) can widen our knowledge on the nature of the cognitive processes at the molecular level despite the integral character and rather large inertia of this parameter in comparison with EEG. Potentially, this continuous-flow method can determine the molecular correlates of recognition and non-recognition in experimental animals; in future, it should assay the difference between the inflow and outflow blood in the human brain. Similar studies can be carried out using positron emission tomography (PET) and/or NMR-spectroscopy.

The study of conscious and subconscious recognition of familiar object and the research of artificially induced cerebral excitation open the vista for comprehensive analysis of the material correlates of intellectual activity at the atomic and molecular levels.

EPR flow dialysis in medicine and experimental biology

Electron paramagnetic resonance (EPR) is a technique based on interplay of electron charge, mass, and the magnetic moment, which is used to detect FR in chemical and biological systems. The examined specimen should be paramagnetic independently on the environment. The EPR-spectroscopy data are widely used in biology, medicine, chemistry, and physics.

The frequency of paramagnetic resonance is proportional to magnetic field intensity. As a rule, EPR is observed at the frequencies of 10^9-10^{12} Hz. At this frequency range, electromagnetic radiation is efficiently absorbed by aqueous media, so the measurements of EPR absorption is carried out in the dried or frozen specimens.

In biology, we deal with aqueous media that can be neither dried nor frozen to maintain life functions, so EPR measurements should be performed at a radically diminished frequency of electromagnetic field and proportionally reduced MF intensity. Nevertheless, the biological effects of FR should be tested at various parameters of EPR. It should be taken into account that the biological processes are characterized by production and recombination of FR and paramagnetic ions, which simultaneously can be the radicals.

In the research, the combined application of EPR and flow electrodialysis can be employed to detect FR and determine the distribution of anion and cation radicals over spectrum bands during artificial circulation. Such a technique is used in the work of artificial kidney in combination with anode and cathode currents to extract the anion and cation radicals in the system employing EPR and NMR with electrodialysis.

The method capable to detect the ions of transitional elements, admixtures in various pathological biochemical reactions, the electrons captured in the regions of radiation damage during radiation sickness, some molecules, and FR should be widely used. Persistent use of EPR in biological objects can change the developing pathology. With some modification, EPR method can be employed as a medical procedure to separate the chemical molecules so that the specific particles detected in the flow can be removed under continuous qualitative and quantitative analysis.

Some new features of the discussed method result from exposure of the specimen to electric field (electrodialysis), which separates the ions that can be FR. However, the following prerequisite condition should be met: both plus and minus poles must be present beside the semipermeable membrane.

Various types of leukemia cells and concomitant infiltrate structures that passed across EPR/NMR flow electrodialysis system should be further examined in tumor cultures to establish their inoculation potency. This method in the system of paramagnetic dialysis can yield new and possibly efficient way to treat leukemia, the consequences of radiation sickness, and other hematologic disorders. The experiments with CMF can be carried out in the cases with ascitic cancer by recording the parameters of ascitic fluid in EPR/NMR flow electrodialysis system.

It is noteworthy that one of the dialysis modes should include interruptive exposure with simultaneous on and off intervals. Probably, such transient arrest of EPR can provoke certain changes in the cascade

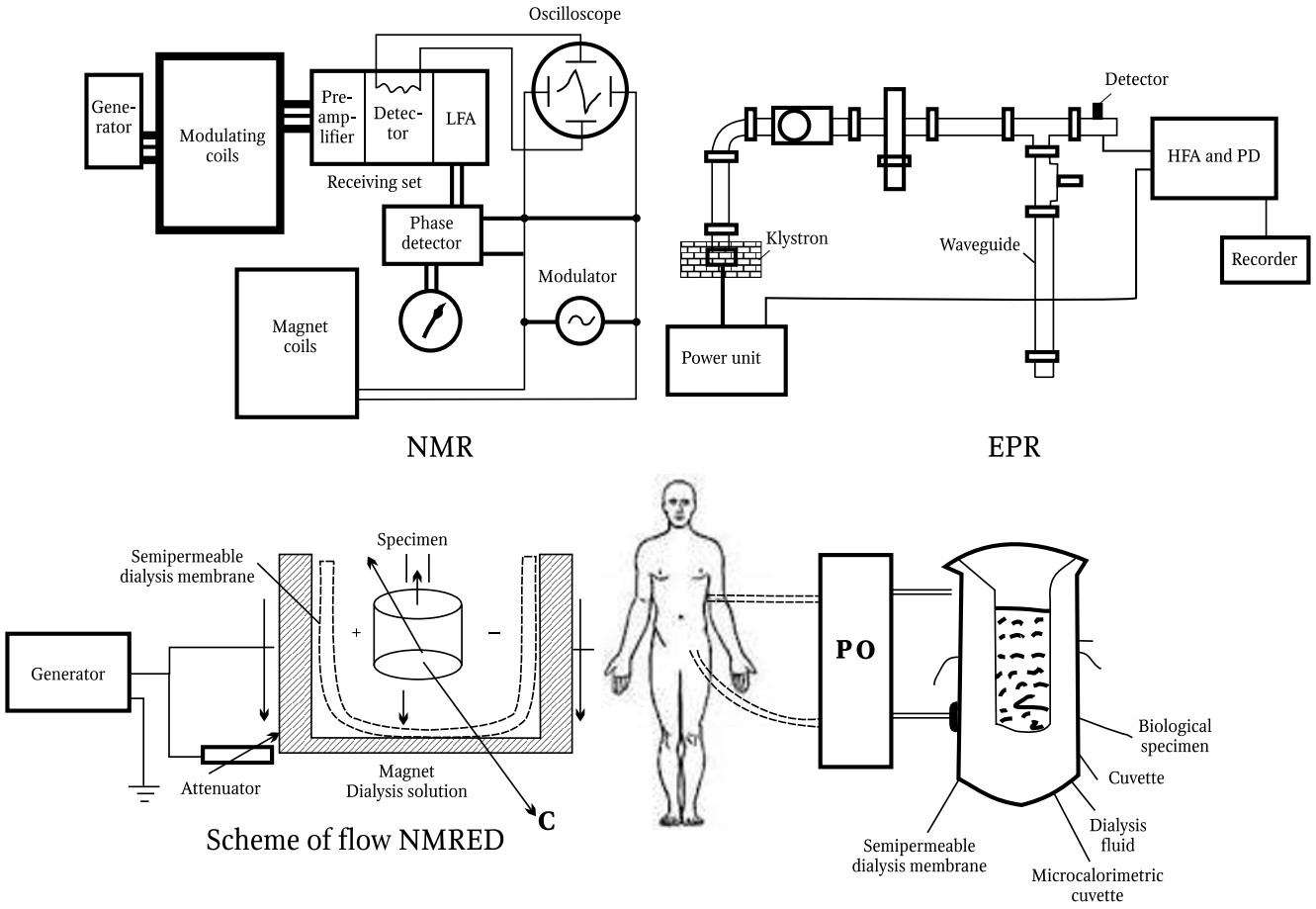

Fig. 1. Experimental scheme of EPR and NMR arrangements coupled with electrodialysis, microcalorimetric system, and pump oxygenator (PO). LFA – low frequency amplifier; HFA – High-frequency amplifier; PO – pump oxygenator.

biochemical processes. An example of this phenomenon is provided by the cultures of synchronously dividing cells [14].

NMR flow dialysis in medicine and experimental biology

One of the important problems in modern medicine and oncology is prevention of postoperative dissemination of tumor. There are certain progress in this field, although further work is needed to develop the methods to minimize tumor metastasizing. Of special interest are the phenomena triggered in the living organism during NMR tomography, which is now used only as a diagnostic tool [16]. In this paper, we discussed feasibility to employ NMR in combination with flow electrodialysis (NMRED). To perform flow electrodialysis, the commercial hemodialysis machines can be used with some modifications needed to apply the electric field between the blood and dialysate.

In 1989, the conception of "racemic lock" was advanced, which implies triggering some biochemical processes in the body resulting from an increase in concentration of a nonspecific stereoisomer derived from the corresponding metabolite [17].

The biological aspects of stereoisomerism can be examined on the blood specimens with the use of NMRED and EPRED methods. In these tests, the biomedical parameters should be chosen to assess efficiency of such assay.

One of the most problematic in natural sciences is the enigma of human organism, so the physicochemical descriptions of magnetobiological phenomena are not entirely correct both medically and biologically. The living organisms possess the magnetic receptors incorporating the biogenic ferromagnetic structures capable to detect the low-intensity magnetic fields.

In human organism, various biological processes involve the paramagnetic and ferromagnetic structures, each playing an individual role in the performance of living organism.

Two questions are logically arising – are the corresponding biomagnetic processes controlled in any way by the geomagnetic field (about 0.5 Gs), and do these paramagnetic, diamagnetic, and ferromagnetic structures play some role in evolution? Probably, the

answers to both questions should be confirmative because of the evident fact that biological evolution developed under the action of geomagnetic field.

The advanced methods of NMRED and EPRED combined with artificial circulation facilities (Fig. 1) will make it possible to implement diagnostics and treatment at the atomic and molecular level such as removal of cytotoxic superoxide radicals from the organism. These methods can be also employed to study the atomic and molecular basis of radiation sickness, oncologic diseases, gerontological diseases, epilepsy, and other pathologies. All these diseases are accompanied by elevation of FR concentration.

Efficiency of diagnostic and therapeutic methods can be assessed only in the process of authorized comprehensive and versatile medical testing. Any biological subsystem gains additional energy in the external magnetic field. With due account for atomic and molecular interaction, the resuscitation procedures needs the development of NMRED and EPRED apparatuses to estimate efficiency of the atomic-molecular prevention and therapy. The modern scientific and technological basis makes it possible to start research and development of such apparatuses followed by their testing in real medical environment under the established order.

REFERENCE DICTIONARY

***1. Electron Paramagnetic Resonance (EPR)** is a resonant absorption of electromagnetic waves caused by quantum transitions in a paramagnetic specimen. EPR was first observed in Kazan State University by Soviet physicist, academician Yevgeny Zavoisky in 1944 (I. I. Silkin. Yevgeny Konstantinovich Zavoisky. Kazan, 2007). EPR spectra are mainly observed within the microwave frequency range and used to examine the solid body structure or to construct the quantum amplifiers. EPR is also used in chemistry, biology (for example, to examine FR), and medicine. It is one of the methods employed in radio-frequency spectroscopy in centimeter and millimeter ranges. The developed EPRED apparatuses are intended to excrete the anion and cation radicals from the organism.

2. Nuclear magnetic resonance (NMR) is one of the radio spectroscopy methods observed when the examined specimen is exposed to two mutually perpendicular MFs: one MF is strong and constant, while other is weak and alternating at the frequency range of 10^6-10^7 Hz. Although NMR is a quantum effect, some features of it (like those of other types of magnetic resonance phenomena) can be explained by classical physics. Most of atomic nuclei possess intrinsic angular momentum J=Ih where I is nuclear spin. Spin underlies the dipole magnetic moment of the nuclei resulting in NMR. The developed NMRED apparatuses are intended to excrete the anion and cation radicals from the organism.

3. Spin is the intrinsic angular momentum of an elementary particle of quantum nature not related to translational motion of the particle as a whole entity. Spin is the additive vector parameter that can describe angular momentum of entire atomic nucleus or atom. In such case, the total spin is a vector sum of individual spins calculated according to the rules of quantum mechanics. Thus, the total spin takes into account the angular momenta of all elementary particles incorporated into the system including the individual spins of these particles and the angular momenta resulting from their orbital motion. Spin is measured in the same units as Plank constant $\boldsymbol{h}$, and it is equal to $\boldsymbol{Jh}$, where $\boldsymbol{J}$ is a characteristic parameter of any type of elementary particles, an integer positive number (zero value included) referred to as "spin quantum number".

4. 1 Tesla=10,000 Gauss.

* "The discovery of Zavoisky is more than matched for Nobel Prize" (Academician Peter Kapitsa).

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