For decades one might observe trend of ESR movement to Biomedical studies. Crude look back at the field might supplement specialized studies. This talk is an attempt to make brief historical overview of this uneasy voyage.

Oleg Grinberg
Outlines of the talk

- EPR/ESR discovered in Physics
- “Chemical” period of EPR/ESR
- High-Frequency EPR in Lebedev’s lab
- Update on Biomed at Dartmouth
  1. MCAO & HBO
  2. Clinical Oximetry
  3. *In-vivo* Dosimetry
- Instead of Summary
Recently I was impressed by the article “Magnetic resonance: discovery, investigations, and applications” PHYSICS-USPEKHI, 52(7), 695-722 (2009), written by Lebedev’s friend Alexander Kessenikh. So kindly invited to this Workshop I was thinking that perhaps it might be interesting to share briefly my view on some events in history of EPR/ESR.

Very soon I recognized that this is extremely difficult task to say something new after Berliner’s V18 “EPR in vivo”.

Thinking of my talk at this Workshop I was surprised that some old studies could be linked to EPR Microscopy.
In 1896 Joseph Larmor published his Larmor's theorem. It was the beginning of magnetic resonance. Very soon after that the interaction of spin with an external magnetic field was discovered by Pieter Zeeman at Leiden University.

Hendrik Lorentz, Wolfgang Pauli, Alfred Lande’, Stern and Gerlach, Ramsey, Einstein and Ehrenfest and many others magnificent physicists created history of Magnetic Resonance.
Interesting fact: Evgenii Zavoiskii was searching for nuclear magnetic resonance (~100 MHz), but discovered (1944) electron paramagnetic resonance (‘Columbus's casus’)

Most surprising is that the EPR discovery was made under the severe conditions in Kazan during wartime.

The setup was restored through the efforts of I. Silkin, the keeper of the Museum-laboratory. Picture was taken by Ildar Salikhov
EPR/ESR becomes very exciting field for researchers. It involves theoretical considerations as well as technical developments. As A Abragam well-aimed noted in the preface to the `bible' of NMR that a specific feature of this field of science consists in a very close connection between theory and experiment, and this leaves no place for a theory that cannot be checked by experiment, or for an experiment that does not have a theoretical interpretation.

ESR, which was discovered in compounds of transition metals, was soon discovered in paramagnetic systems of a different nature. In 1949, EPR was observed for the first time in crystals with radiation damage and from 1947 to 1950 in free radicals. Chemists became possessors of very powerful tool.
Voevodskii’s (VV) lab

- Academician V.V. Voevodskii was among the first ones who had understood the importance of EPR/ESR for Chemistry. The basic direction of his activity became the research of the structure and properties of free radicals in varied chemical reactions by means of ESR.

- For the USSR authorities the goal of the research at that time was pronounced as a development of fuel for missiles using free radicals. This allows officially establishing the lab in 1955.

- Therefore one of the first publication at this time was: Investigation of free radicals formed in solids subjected to bombardment by fast electrons, Molin YN, Koritsky AT, Buben NJ, Voevodsky VV, Doklady Akademii Nauk SSSR: 123 (5) 882-883 (1958)
I joined VV’s lab in 1965 as a student, when EPR/ESR method was already widely employed for the investigation in chemistry. The most exciting to me topic at that time was Radical Pairs, especially created by UV-light.


- Selective generation of radical pairs in benzoyl peroxide single-crystals irradiated by polarized UV light, Barchuk, VI; Dubinsky, AA; Grinberg, OY and Lebedev YS, Chemical Physics Letters 34 (3) 476-478 (1975).

In this studies we were able to generate radical pairs of different orientation by varying orientation of polarized light
Voevodskii did not live to 50-year age. The tool, EPR Spectrometer for chemical studies, was developed in his lab.

The school of outstanding researchers in the field of ESR he created is the most important VV’s heritage

After Voevodskii death Lebedev flew to Novosibirsk to meet Tsvetkov and Molin. They discussed where to go for future development of EPR/ESR/NMR: Molin was going to focus on NMR, Tsvetkov – on Pulsed techniques and Lebedev on further development of CW EPR.
Lebedev returned home full of ideas. Very soon after this meeting he offered his graduate students to develop theoretically three projects:

- Dubinskii supposed developing Fourier EPR Spectroscopy by analogy to Far Infrared Spectroscopy. It was too early for such project. It was later went to Pulsed FT ESR.

- Yakimchenko began working on ESR Imaging. First micro imaging at that time were derived using field gradient created by magnetic needles. This approach is used in ESR/AFM.

- I guess I was lucky. I was offered working on High-Frequency EPR. My goal was to define the highest technically achievable frequency at that time. It was very exciting and uneasy job.
The first version of D-band (2mm) EPR spectrometer was in operational conditions in 1974. However the multi modes resonator used in this device created unacceptable difficulties: lineshape was a function of tuning and coupling. I think now that it could be used for some kind of ESR Microscopy. But in that time we have not though on this direction. The second version of D-band spectrometer (1976) with TE011 cavity was perfect. High spectral resolution and sensitivity 5*10^7 spin/G. We became possessors of the unique tool for many years.

High spectral resolution in High frequency ESR allowed
a. Reliably to determine the magnetic parameters of radicals in amorphous samples
b. Investigate effects of radical structure on g-values and vice versa
c. Observe effects of the interaction of the radicals with environment

Molecular motion by High frequency ESR allows to extend interval of experimentally measurable correlation times in the fast motion domain by approximately an order of magnitude compare with the X-band

In several experiments we observed spectra that we were unable to explain by conventional model

Also at that time we began to pay more attention to biological systems
a. We believed that it is future needs
b. The MRI method became essential in medicine
We were impressed by detection of mitochondrial respiration performed in Molin’s lab [Molin et. al (1976)]. This study was performed using 100 liver cells. We naively assumed that we would be able to detect respiration by a single cell.

However our attempts to investigate biological systems like cell suspensions or protein solutions by High Frequency EPR met significant difficulties: the diameter of sample tube for lossy solution should be less than 0.1mm. However we were unable to put any biological sample inside of such a small tube.

Therefore 3rd version of D-Band EPR Spectrometer using Fabry-Perot resonator was especially dedicated to biological systems. However “Perestroika” began.
Occlusion of an artery in the brain leads to the development of an ischemic core and penumbra. The ischemic core is the area where blood flow is reduced to <15% of normal. The ischemic penumbra is the area where blood flow is reduced to between 40% and 15% of normal. The fate of the penumbra is dependent upon key events that determine life or death for cells in this region, since cells in this area are in danger but not yet irreversibly damage.
Hyperbaric Oxygen Therapy (HBO) potentially may improve clinical outcomes

Acute Ischemic Stroke → Ischemic and Reperfusion Cascade

- ATP Depletion
- Glutamate & Calcium Imbalance
- Depolarization
- Mitochondrial Damage
- Oxidative Stress
- Inflammation
- Apoptosis

HBO Therapy

- Improved Oxygen Delivery and Oxygen Extraction
- Improve Metabolism
- Stabilize Glutamate, Glucose
- Inhibit Inflammation
- Enhance Superoxide Dismutase
- Enhance Bcl-2
- Decrease Deformability of Red Blood Cells

Brain Infarction
Brain Edema
Blood-Brain Barrier
Mortality
Neurological Deficit

Reduce Mortality
Reduce Neurological Deficit

1. Anatomic positions of the LiPc implants and focal ischemia
2. MCAO model in rats
3. EPR oximetry and HBO therapy
Recent small group study, experimental protocol

Male Sprague-Dawley rats (250g - 310g) [N =3-5]

- LiPc Implantation 5-7 days earlier
- Male Sprague-Dawley rats (250g - 310g) [N =3-5]
- Ischemia pO₂
- Baseline pO₂
- Intervention pO₂
- Reperfusion pO₂
- TTC staining, and infarct size
- Recover pO₂
- ~24 hours
- ~5 hours
- Reperfusion Initiated
- Intervention (HBO, 100%, or 30% O₂)
- Occlusion Initiated
- Ischemia
- Ischemia pO₂
- Surgical Prep
- Ketamine
- 1 hour
- .5 hour
- .5 hour
- Baseline
- Ischemia
HBO intervention is capable of elevating pO₂ in at-risk tissue on the periphery of the infarct volume.

Treatment with HBO during occlusion reduces infarct volume.
Mean $pO_2$ reflects effects of HBO. However more rigorous analysis is required.

$N_{30\%} = 5$

$N_{100\%} = 8$

$N_{HBO} = 5$

Results are mean ± SEM.
Clinical EPR Oximetry studies currently ongoing at Dartmouth:

- Superficial Tumors, to optimize hypofractionated radiotherapy by irradiating at times of optimal tumor oxygenation
- Peripheral Vascular Disease

The goals for the initial clinical tumor measurements included:

- Refinement of the methodology needed to make measurements of tumor pO$_2$ in human subjects under conditions that are compatible with the constraints of clinical practice and comfort of patients
- Verification that the measurements can be accomplished in tumors in human subjects
- The performance of serial measurements of tumor pO$_2$ in human tumors during the course of radiation therapy and in response to hyperoxygenation
EPR Oximetry: Procedures

Patient preparation - Injection of India ink

- India ink formulated with Printex-U carbon black (200mG/mL) in 0.9% NaCl and 1.6% CMC
- Sterilized via autoclave prior to injection
- 20-50\(\mu\)L injected into tissue of interest using 22 gauge needle
  - Visual and tactile guidance
  - Optional local anesthetic (lidocaine)
  - 2-10mm depth
- Short recovery period (1-2d) is typically allowed prior to measurement

Measurements

- Clinical whole-body spectrometer with temperature control
- EPR data collection (1-5 min/set) averaging and over the course of treatment, 3-5 min/set
- Measurements repeated as desired
  - Continuous measurements of \(O_2\) dynamics
  - Acute Pre- and post- therapy
  - Over the course of treatment
Measurements performed for 12 subjects

- melanoma lesions and metastases, basal cell, soft-tissue sarcoma, and lymphoma tumors.

(left) The cosmetic result of ink injection is a bluish dot, whose size ranges from (a) 0.5 mm for deeper tissues to (b) 5 mm for superficial tissues.
EPR spectra and tissue pO$_2$ within a melanoma lesion with volunteer breathing air and the response to breathing 100% O$_2$. Measurements with air and 100% were averaged for 2.5 and 5 minutes respectively, and the mean interval between measurements was approximately 8 minutes.
EPR Oximetry: Hyperoxygenation

Oximetry results for a melanoma metastasis and the response to increased fraction of inhaled O₂.

EPR spectra, and associated spectral fitting, acquired (a) immediately prior to O₂-breathing, (b) after 10-min of O₂, and (c) after returning for 4-min to air-breathing. The decrease in amplitude and slight increase in the linewidth are indicative of the increase in tumor pO₂.

pO₂ values averaged over 50-sec periods are shown for the experimental period (mean±SE, n=5), indicating a small (~3mm Hg) but robust change in the tumor pO₂ resulting from inhaled O₂.
Tumor pO₂ was monitored in melanoma metastases at two sites, in the scalp and neck, during the course of radiation treatment. Spectra were recorded immediately before and after each fraction while the patient inspired room air. These results indicate hypoxic environments that vary on a day-to-day basis, but show little acute response in pO₂ due to radiation.
Technical challenges have been overcome through varied preliminary measurements.

Further optimization of injection for both EPR and cosmesis underway.
- Transdermal & Intra-operative
Future Clinical Applications

**NCI-PPG proposal under review**

- Implantable resonators have been developed and testing in animal models
  - Increased sensitivity
  - Increased measurement depth
- Optimized biocompatible O₂-sensors
- Proposed studies include:
  - Breast, prostate, cervix tumors during RT
  - Post-RT and post-surgical tumor bed
  - tissue flap reconstruction

**Additional application in wound healing and vascular disease.**
**Consequences of irradiation:**

- (<50 cGy) - no acute symptoms
- (<150 cGy) - mild and delayed symptoms
- (<300 cGy) - moderate to severe clinical symptoms
- (≥300 cGy) - early severe clinical symptoms with potential for lethal outcome
- (≥600 cGy) - very likely to lead to early death

**Overall Aim:** Development of a method to rapidly identify significantly exposed individuals and estimate absorbed dose to enable effective emergency care.
EPR Biodosimetry

- **In vivo Tooth Dosimetry**
  - Production of an FDA approved mass-producible device
  - Collaboration with General Electric
  - Establish precision of strictly \( \leq 0.5 \text{ Gy} \)
  - Sensitivity and Specificity \( \geq 95\% \) at 2 Gy threshold
  - Reduce further the impact of interpersonal variability
  - Validation through collaboration with Dana Farber for TBI dosimetry

- **Clipped Nail Dosimetry**
  - Further reduce variability and impact of mechanical signals
  - Assess demographic and personal variables
  - Complete developments for field-use and non-expert operators

- **In vivo Nail Dosimetry**
  - Further optimization of surface resonators to increase sensitivity
  - **In vivo** measurements, including TBI populations
  - Complete developments for field-use and non-expert operators
EPR Tooth Dosimetry

Isolated teeth

Inserted Teeth

Mouth Models

Normal Subjects, Irradiated Patients

Finite Element Modeling

In vivo EPR dose response for lower canine teeth during the RT, 1 Gy/fraction.
In-vivo Tooth Dosimetry

- Sensitive to x, γ, unaffected by dose rate and provides output immediately
- Physical process not affected by injury
- Non-invasive and field deployable
- Complementary with other assays
- Measurements can be made at any time after irradiation
- Use by minimally trained individuals
- Acceptable cost per measurement

- Requires size and kind of tooth correction
- Effect of UV, tooth bleaching etc.

Recent measurements on TBI patients suggest that the current instrument and procedures enable dose estimation with a standard deviation of 1Gy
**Instrumentation Evolution**

**Original clinical instrument**
- L-band (1.2 GHz)
- 420 Gauss permanent magnet
- Surface loop resonators designed for intra-oral use
- Articulating resonator mount

**Current Dosimeter**
- Fully transportable
- Self-contained compact electronics and display unit
- Small permanent magnets

**Future?**
- FDA approved
- Mass produced
- Kiosk-based
- Fully integrated components
- Comprehensive automation of resonator positioning and data acquisition.
Field Deployment Exercises

- May 2008 Clin. Lab
- July 2009 Loc. Field
- May 2009 Loc. Field
- July 2010 Loc. Field
- July 2010 Remote Field
In Vivo Fingernail Resonator

- Developments in process in collaboration with MCW
- Retains benefits of the clipped nail approach
- Avoids generation of mechanical signal
- Localized $B_1$ interacts only with the nails and not the underlying soft tissues

Surface Resonator Array

Sub-wavelength Aperture Resonator

MIS EPR signal in human nails at AR based on TE102 cavity

Graph: Magnetic field, G vs. SA, au
It is well known that the success could be achieved if a goal is reinforced by available tool.

From another hands very often it is uneasy to identify our resources in advance. For example it is known that in 1938-42 Gorter and colleagues were in very good position to observe ESR. However they even did not attempt to observe ESR because the EPR lines were expected to be too wide.

I wish all of us that our desires and our opportunities would coincide!
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