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Electron paramagnetic resonance studies of free radicals: The research legacy of Janko N. Herak

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ABSTRACT

This review presents a chronological analysis of the scientific contributions of Professor Janko N. Herak (1937–2025), a Croatian biophysicist whose research played a major role in the development of electron paramagnetic resonance (EPR) spectroscopy in Croatia and significantly advanced the study of free radicals in biological systems. Beginning with his early work at Duke University under the mentorship of Prof. Walter Gordy, Herak investigated radiation-induced radicals in nucleic acid bases, nucleosides, and related biomolecules using EPR spectroscopy.

During subsequent decades at the Ruđer Bošković Institute and later at the University of Zagreb Faculty of Pharmacy and Biochemistry, he conducted extensive studies of free radicals in irradiated single crystals of nucleic acid constituents, elucidating mechanisms of radical formation, hydrogen transfer, and structural rearrangements. His research later expanded to the investigation of oxidative processes in biological macromolecules, particularly the slow oxidation of plasma lipoproteins, where EPR spectroscopy and kinetic modelling provided insight into radical-mediated lipid peroxidation and the physicochemical basis of lipoprotein modification.

In parallel, Herak explored long-range charge migration in nucleobase crystals and investigated metal-nucleobase interactions, integrating experimental EPR and ENDOR spectroscopy with theoretical modelling. These studies contributed to a deeper understanding of radical stabilisation, electronic structure, and energy transfer in ordered molecular systems.

By situating Herak's work within the broader development of radiation chemistry and magnetic resonance spectroscopy, this review highlights the methodological rigour, conceptual contributions, and lasting scientific impact of his research on free radicals and biologically relevant molecular systems.

Keywords: electron nuclear double resonance (ENDOR), electron spin resonance (ESR), free radicals, nucleic acid radicals, lipoprotein oxidation, long-range charge transfer, metal-nucleobase interactions

INTRODUCTION

Electron paramagnetic resonance (EPR) spectroscopy has played a central role in the investigation of free radicals and radiation-induced processes in chemical and biological systems. Among the scientists who contributed significantly to the development of this

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field in Croatia was Full Prof. Janko N. Herak (1937–2025), whose research spanned more than four decades and encompassed fundamental studies of radical formation, molecular structure, and oxidative processes in biologically relevant systems.

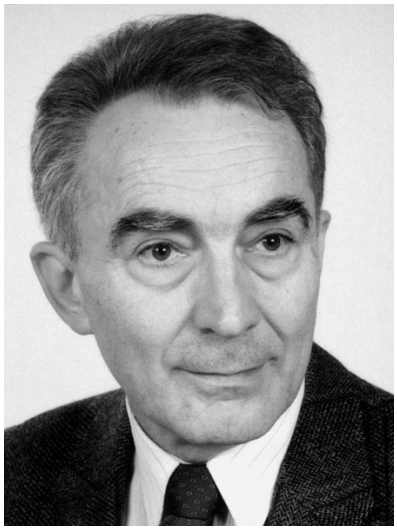

Fig. 1. Professor Janko Nikola Herak

Prof. Herak's scientific career combined experimental spectroscopy, radiation chemistry, and theoretical analysis. His early research focused on the identification and structural characterisation of radicals formed in nucleic acid bases and related biomolecules exposed to ionising radiation. Through detailed EPR and electron nuclear double resonance (ENDOR) studies of irradiated single crystals, he helped establish nucleic acid model systems as powerful tools for investigating radical formation, hydrogen transfer, and radiation damage at the molecular level.

In later stages of his career, Herak expanded his research toward biologically complex systems, particularly the oxidative modification of plasma lipoproteins. Using EPR spectroscopy and kinetic modelling, he investigated the mechanisms of lipid peroxidation and the role of transient radical species in the slow oxidation of low-density and high-density lipoproteins. Parallel studies addressed long-range charge transfer processes in nucleobase crystals and the electronic structure of radical species through combined spectroscopic and computational approaches.

Beyond his scientific publications, Prof. Herak played a key role in establishing EPR spectroscopy in Croatia, including the construction of the very first domestic EPR spectrometer and the mentoring of several generations of researchers in EPR spectroscopy.

The aim of this review is to present a structured overview of Prof. Herak's scientific contributions, emphasising the evolution of his research interests and the broader significance of his work in the fields of radiation chemistry, biophysics of nucleic acids, free radical chemistry, and EPR spectroscopy.

DUKE UNIVERSITY – DEPARTMENT OF PHYSICS (1966–1967): TRAINING IN EPR SPECTROSCOPY UNDER PROF. WALTER GORDY

The earliest phase of Janko N. Herak's scientific career took place at the Department of Physics, Duke University, where he conducted pioneering work in EPR spectroscopy under the mentorship of Prof. Walter Gordy, one of the founders of molecular EPR spectroscopy. During this period, Herak became involved in experimental and theoretical studies of molecular structure and rotational spectra, acquiring a strong foundation in spectroscopic methods and physical chemistry that would later prove essential for his subsequent work in EPR spectroscopy. The research carried out during this stage of his career focused primarily on the EPR spectra of small molecules and radicals, with particular attention to the determination of molecular structure and spectroscopic constants. These studies, published in a series of papers during the early phase of his career, illustrate Herak's early engagement with high-precision spectroscopic techniques and his growing interest in the structural interpretation of spectroscopic data.

In his first study carried out in collaboration with Prof. Gordy, Herak investigated the formation of free radicals produced by atomic hydrogen bombardment of several nucleic acid bases, including thymine, uracil, adenine, and guanine (1). Using EPR spectroscopy, the authors demonstrated that hydrogen atoms can add directly to specific carbon atoms of the heterocyclic rings, namely purine and pyrimidine, giving rise to well-defined radical species characterised through their proton hyperfine structures. These results provided clear experimental evidence for hydrogen-addition reactions in biologically relevant molecules and contributed to the understanding of radiation-induced radical formation in the constituents of nucleic acids.

In a subsequent study, Herak investigated the electron spin resonance spectra of gamma-irradiated powdered anhydrous α-maltose to characterise radiation-induced radical centres in this carbohydrate (2). Analysis of the spectra, combined with temperature annealing experiments, revealed the presence of at least three distinct types of damage centres with different kinetic behaviours and stabilities. The results suggested that the observed radicals originate from specific bond cleavages, including rupture of the C–H bond at C1 and possibly the glycosidic bond, providing insight into the mechanisms of radiation damage in carbohydrate structures.

Following his studies on radiation-induced radicals in carbohydrates, Prof. Herak extended his focus to nucleic acids, investigating how structural features influence radical formation. While the maltose experiments had highlighted the role of specific bond cleavages and radical stability in small biomolecules, he now explored similar processes in the more complex polymers such as RNA and DNA molecules, moving from single nucleic acids. Using low-temperature hydrogen and deuterium atom bombardment combined with EPR spectroscopy, he was able to reveal temperature-dependent radical formation in RNA bases, including cytosine, which was previously unreactive at room temperature, while DNA remained largely unreactive under the same conditions (3). This progression from simple carbohydrate models to nucleic acid polymers illustrates Herak's systematic approach to understanding the interplay between molecular structure and radical chemistry.

Extending their prior studies on nucleic acid bases, Herak and Gordy investigated the formation of hydrogen-addition radicals in nucleosides and nucleotides. Using gaseous H

and D atoms at thermal energies and observing EPR spectra at temperatures ranging from 77 K to 770 K, they demonstrated that H-addition occurs readily on nearly all nucleosides and nucleotides except those derived from adenine (4). Specifically, radicals were consistently observed on guanine and thymine derivatives, whereas the uracil and cytosine rings displayed temperature-dependent patterns, reflecting differences in steric accessibility and local molecular environment. These results provide strong evidence that the three-dimensional arrangement of DNA prevents external hydrogen atoms from accessing reactive sites, a shielding effect that is not present in RNA or in isolated nucleosides and nucleotides. Moreover, the study confirmed that previously observed radical signals in RNA and free bases correspond to direct H-addition, reinforcing the mechanistic insights derived from earlier base-focused studies.

In the subsequent investigation, Herak and Gordy employed EPR spectroscopy on homopolymers and copolymers of nucleotides, demonstrating that hydrogen atoms liberated from bound water can add to nucleotide bases, generating H-addition radicals, whereas radicals in polymers such as cytosine polynucleotides (Poly C) were largely unstable (5). The study revealed that hydration (*via* H_2O or D_2O) markedly influences both the formation and stability of these radicals, whereas copolymeric polynucleotides indicated the relative susceptibility of nucleotides to radiation in the order C > U > A. These findings underscore that hydrogen radicals derived from water serve as a significant source of indirect damage to nucleic acids, with radical instability at elevated temperatures potentially resulting in either restoration of the original ring structures or permanent modification of the bases.

Herak and Gordy subsequently investigated the direct interaction of O–H radicals with uracil at ambient temperature, revealing addition at the C5 ring position (6). EPR analysis showed migration of the original C5 hydrogen to the C6 position, creating a methylene group with two equivalent proton couplings at C5. These radicals were generated by exposing powdered uracil to a low-velocity O–H radical beam derived from hydrogen peroxide or water vapour under reduced pressure with an electric discharge.

Building upon their previous work, Herak and Gordy finally investigated the reactivity of powdered uracil derivatives toward gaseous hydrogen atoms, identifying the resulting radicals *via* EPR spectroscopy (7). Their results demonstrated that in 5-nitrouracil and uracil-5-carboxylic acid, the NO_2 or COOH groups were initially substituted by hydrogen to regenerate uracil, which then underwent hydrogen addition. Similarly, the COOH group in uracil-6-carboxylic acid and the NH_2 group in 6-aminouracil were replaced by hydrogen atoms, whereas 5-aminouracil, uracil-5-sulfamic acid, and 5-acetyluracil primarily formed direct hydrogen-addition radicals at C5, with minor substitution. For 6-methyluracil, only direct C5 hydrogen-addition radicals were observed, and proton couplings for all radicals were quantitatively determined.

RUĐER BOŠKOVIĆ INSTITUTE (1969–1971): PIONEERING EPR STUDIES IN CROATIA

After returning to Croatia in 1968, Prof. Herak, in collaboration with engineer Vladimir Galogaža, constructed one of the first EPR spectrometers in this part of Europe, and the very first in Croatia, then part of the former Yugoslavia. This instrument enabled the initiation of research on free radicals in biological macromolecules and supramolecu-

lar structures at the Ruđer Bošković Institute. The tradition of domestically built spectrometers, established by Herak and Galogaža, continued for many years, encompassing successive generations of instruments in the same laboratory.

The first research on the newly constructed EPR spectrometer in Croatia focused on single crystals of cytosine monohydrate, irradiated and analysed at 77 K, where two radical species, cation and anion, were detected and analysed in detail (8). Later studies at room temperature revealed at least one additional radical formed by hydrogen atom addition on the pyrimidine ring, with hyperfine analysis indicating that the unpaired electron was centred on C4 and the hydrogen atom added at C5 (9). Furthermore, careful examination of multiple crystal orientations confirmed the presence of two magnetically distinct molecules, and no significant molecular rearrangement occurred upon irradiation, highlighting the precision and sensitivity of the early EPR measurements.

Soon thereafter, attention shifted to the study of radical transformations in DNA and its constituent bases using EPR spectroscopy (10). It was observed that, upon warming irradiated samples at 77 K, hydrogen-addition radicals consistently formed in all bases. Based on these observations, a mechanism was proposed whereby initially damaged species, likely ion radicals, release hydrogen atoms upon warming, which subsequently add to undamaged bases, preferentially targeting thymine. Importantly, thymine does not necessarily correspond to the site of primary radiation-induced damage, highlighting the dynamic nature of radical migration and recombination in nucleic acid crystals.

The research on radical transformations was further extended to the study of hydroxyl-group abstraction by thermal hydrogen atoms (11). Using powdered alloxan and single crystals, Herak demonstrated that hydroxyl groups can be selectively abstracted, producing C-centred $R_1(R_2)\dot{C}$-OH radicals, as confirmed by EPR spectroscopy. The results suggested that the reaction involves the formation of an unstable complex between the hydrogen atom and the excited-state hydroxyl group, which subsequently yields the radical and a water molecule, highlighting a previously unobserved pathway for radical formation in organic solids.

Building on his earlier studies of radicals in nucleic acid bases, Herak extended EPR investigations to deuterated single crystals of thymidine to elucidate the behaviour of hydrogen-addition radicals (12). Irradiation at 77 K revealed radicals formed by deuterium addition at C6 of the thymine ring, and subsequent warming induced a deuteron-proton exchange, which occurred intermolecularly without affecting radical concentrations, highlighting the mobility and transformation of hydrogen atoms within nucleic acid constituents. These observations suggested potential biological implications, as the displacement of hydrogen atoms from thymine could disrupt base pairing with adenine. Complementary studies on gamma-irradiated dihydrouracil identified radicals of the $C5H_2$–C6H type and revealed two magnetically distinct molecules (13). Analysis of the α- and β-proton couplings indicated that the N6 atom moves into the molecular plane following hydrogen abstraction from C6, while C5 remains out of plane, consistent with calculated C–H bond energies showing H6′ as the most easily removed hydrogen. Collectively, these investigations provided detailed structural insight into radical formation, hydrogen migration, and the stereochemical consequences of irradiation in nucleic acid bases.

Extending his investigations on nucleic acid bases, Dulčić and Herak applied EPR spectroscopy to study radiation damage in single crystals of thymine, both monohydrate and anhydrous forms (14). The formation of 5-thymyl radicals was observed in both crystal

systems, demonstrating that thymine molecules can act as both donors and acceptors of hydrogen atoms. Detailed analysis of the methyl and methylene proton couplings revealed that the electronic structure and configuration of the 5-thymyl radical are essentially independent of the crystal form, with the pyrimidine ring adopting a distorted conformation around C5 and C6. These findings confirmed that hydrogen abstraction and addition involve intramolecular and intermolecular processes, altering the C5–C6 bond length and causing out-of-plane displacement of C6, while other bonds remain largely unaffected. Altogether, these studies provided precise insight into stereochemistry, hydrogen migration, and radical conformations in thymine, further illustrating the general principles of radical formation and transfer in nucleic acid bases.

Herak and Schoffa also investigated radiation-induced radicals in single crystals of 6-azathymine using EPR spectroscopy to clarify the possibility of hydrogen abstraction from the methyl group (15). Analysis of spectra from both non-deuterated and deuterated crystals demonstrated that irradiation produces radicals formed by the abstraction of a hydrogen atom from the methyl group, yielding a CH_2 radical conjugated with the pyrimidine ring. Hyperfine interactions with two equivalent protons and two nonequivalent nitrogen nuclei revealed significant delocalisation of the unpaired electron over the molecular framework. These results provided unambiguous experimental evidence for methyl-group hydrogen abstraction in substituted pyrimidines, supporting earlier interpretations proposed for thymine and 5-methylcytosine radicals.

RUĐER BOŠKOVIĆ INSTITUTE (1972–1976): MENTORING FIRST DOCTORAL STUDENTS AND ADVANCING EPR RESEARCH ON FREE RADICALS

Between 1972 and 1976, Professor Herak supervised his first generation of doctoral students, Antonije Dulčić, Boris Rakvin, and Dubravka Krilov, who would later emerge as prominent researchers and internationally recognised experts in EPR spectroscopy. This period marked the beginning of Prof. Herak's influential role as a mentor, during which the supervision of these doctoral theses fostered a productive research environment and resulted in a substantial number of high-quality scientific publications.

Further EPR investigations of gamma-irradiated single crystals of thymine, initiated in 1971, both in monohydrate and anhydrous forms, revealed radicals formed by the abstraction of a hydrogen atom from a nitrogen site (16). Analysis of hyperfine interactions with the methyl protons and the nitrogen nucleus showed that the coupling tensors and *g*-tensor parameters were essentially identical in both crystal systems, indicating that the structure of the H-abstraction radical is determined by the molecular properties of thymine rather than by the crystal environment. Molecular orbital calculations suggested that the abstraction most likely occurs at the N1 position, producing a radical in which the unpaired electron is partially delocalized over the pyrimidine ring. These findings further demonstrated that thymine could act both as a donor and acceptor of hydrogen atoms under irradiation, providing additional insight into the mechanisms of radiation-induced damage in nucleic acid bases.

Under Herak's continued exploration of radiation effects in nucleic acid bases, subsequent EPR studies at 77 K revealed that gamma-irradiation of single crystals of thymine (17) and 1-methyluracil (18) produces radicals predominantly in paired forms. Each pair

involves either hydrogen abstraction from the methyl group or hydrogen addition, with radical separations of only a few Ångströms. Analysis demonstrated that the observed triplet states can be explained solely by dipole-dipole interactions without invoking unusually strong exchange couplings, and the results provided mechanistic insight into energy localisation and hydrogen transfer between neighbouring molecules, further highlighting pathways relevant to radiation-induced DNA damage. Following these investigations, two pioneering ENDOR studies extended Herak's work by providing precise measurements of proton couplings in gamma- and X-ray–irradiated nucleic acid derivatives. In the first study on 1-methyluracil, ENDOR resolved couplings of protons located in up to seven surrounding molecules in the host crystal, demonstrating that intermolecular spin interactions are predominantly dipolar and well described by the point-dipole approximation, while intramolecular couplings exhibit significant isotropic components due to delocalisation of the unpaired electron (19). In the second study on minority radicals in X-irradiated thymidine single crystals at room temperature, ENDOR proved essential in isolating radicals constituting less than 5 % of the total concentration, which are formed by hydrogen abstraction from the methyl group, and allowed determination of precise coupling tensors for the affected protons H6 and H7, underscoring the method's capability to resolve subtle radical species otherwise obscured in conventional EPR spectra (20).

Extending these investigations, Herak also examined radiation-induced radicals associated with impurities in gamma-irradiated single crystals of cytosine monohydrate using EPR spectroscopy (21). The study demonstrated that previously unassigned anisotropic doublets originate from sulfur-containing impurity species, most plausibly thiocytosine molecules substituting cytosine in the crystal lattice, with the unpaired electron localised in a C–S related π-radical. Quantitative dose-response analysis further showed that EPR is sufficiently sensitive to detect sulfur impurities at concentrations of only a few parts per million, highlighting the method's capability for identifying trace impurity radicals and characterising their electronic structure in molecular crystals.

Continuing these studies of radiation effects in nucleic acid constituents, Herak and Krilov employed EPR spectroscopy to investigate gamma-irradiated single crystals of deoxycytidine 5′-monophosphate monohydrate (22). Analysis of the hyperfine structure and angular dependence of proton couplings enabled identification of the =C4′–C5′–$H_2$5′ radical formed by cleavage of the sugar-phosphate bond, providing what was considered the first EPR evidence for radiation-induced scission of this linkage in a nucleotide crystal. The results demonstrated that EPR spectroscopy can directly detect primary radicals associated with backbone damage in nucleic acids, thereby contributing to the understanding of the molecular mechanisms of radiation-induced DNA degradation. Following the EPR investigation, subsequent ENDOR studies of gamma- and X-irradiated deoxycytidine-5′-phosphate focused on radicals stabilised at room temperature and revealed the presence of at least three radical species despite the complexity of the EPR spectra (23). One of these radicals originates from the previously identified primary radical through decomposition of the furanose ring and involves couplings with the H5′, H′5′, and H4′ protons, whereas in another, the unpaired electron is predominantly localised at C1′ of the deoxyribose moiety, as indicated by relatively isotropic couplings of the β-protons.

A shift from studies of radiation-induced radicals in nucleic acid constituents became evident in the mid-1970s, when Herak turned toward investigations of magnetic phenomena in solid-state systems and surface structures. In collaboration with Rakvin, EPR

spectroscopy was applied to small NiO particles, revealing superparamagnetic behaviour of antiferromagnetic nanoparticles and demonstrating that their temperature-dependent susceptibility is governed by relaxation over anisotropy barriers, with thermal treatment leading to an irreversible transition to a paramagnetic state through diffusion of nickel ions to anionic vacancies (24). In parallel, proton magnetic resonance studies of hydroxyl groups on silica gel combined experimental measurements with theoretical analysis based on the method of moments, establishing quantitative relationships between resonance linewidths, spectral moments, and the surface density of hydroxyl groups, thereby extending Herak's research toward magnetic resonance investigations of surface and condensed-matter systems (25).

FACULTY OF PHARMACY AND BIOCHEMISTRY (1976–1981): EPR STUDIES ON FREE RADICALS IN NUCLEIC ACID BASES AND ANALOGUES

After moving from the Ruđer Bošković Institute to the University of Zagreb Faculty of Pharmacy and Biochemistry, Prof. Herak continued his investigations of free radicals in nucleic acid bases and their analogues. These studies were carried out using a Varian E-109 EPR spectrometer located at the Ruđer Bošković Institute, which at the time was jointly shared between the two institutions.

In this phase of Herak's scientific investigations, subsequent studies focused on the detailed characterisation of radiation-induced radicals in cytosine monohydrate, employing both EPR and ENDOR spectroscopy to provide complementary structural and kinetic insights. Herak's work initially addressed the identification of the stable radical formed by hydrogen addition to the O2 position of cytosine, elucidating all intramolecular proton couplings and several key intermolecular interactions (26). Using single crystals grown from both H_2O and D_2O solutions, he was able to assign hyperfine tensors to specific protons, distinguishing conformational variants of the radical and clarifying discrepancies in earlier EPR-based assignments. Further investigations examined the thermal recombination of cation and anion radicals within the crystal lattice, revealing stepwise mutual neutralization processes with multiple distinct activation energies (27). The results demonstrated that the Coulomb interaction between closely spaced ionic radicals significantly modulates their recombination kinetics, providing new insights into the spatial distribution and stabilization mechanisms of radiation-induced radicals in a hydrated nucleobase. Collectively, these studies established a comprehensive understanding of radical formation, stabilization, and recombination in cytosine monohydrate, forming a pivotal component of Herak's early contributions to the field of radiation chemistry and EPR spectroscopy.

In the continuation of his investigations, Herak's scientific research encompassed detailed EPR studies of radiation-induced radicals in the sugar-phosphate region of nucleotides. Gamma irradiation of single crystals of uridine 5'-phosphate (Na salt) at 77 K led to the formation of a PO_4^{2-} radical through scission of the C5′–O5′ bond (28). EPR analysis revealed a nearly axially symmetric g-tensor and isotropic ^{31}P hyperfine coupling, indicating that the unpaired electron resides on the phosphate group; the radical was unstable above 100 K. In parallel, deoxycytidine 5'-phosphate crystals produced an unstable $\dot{O}–CH_2–R$ type radical, with a highly anisotropic g-tensor and β-proton couplings of a typical value of 8.0 G (29). Spin density calculations confirmed the observed couplings, while the lack of hydrogen bonding explained the relatively small β-proton interactions. These studies

together provided a clear picture of phosphate radical formation in nucleotides and highlighted how molecular and crystalline environments influence both the location and stability of unpaired electrons.

Further investigations by Herak and colleagues explored minority and ionic radical species in nucleic acid constituents and pyrimidine crystals. EPR studies of gamma-irradiated single crystals of uridine 5'-phosphate (sodium salt) revealed the presence of NO_2 molecules in minor quantities, originating from impurities in the crystal (30). The occurrence and EPR signatures of these radicals depended on the commercial source of the sample, confirming that they were not induced by irradiation of the nucleotide itself but rather associated with nitrate or nitrite impurities. These NO_2 species accounted for approximately 1 % of the total paramagnetic centres and displayed characteristic 1:1:1 triplet EPR patterns corresponding to the coupling of the unpaired electron with a ^{14}N nucleus. Parallel studies on isocytosine single crystals demonstrated that gamma irradiation induced multiple ionic radical species, including a cation radical localised predominantly on tautomer A, an anionic doublet radical likely formed *via* proton transfer to the anion, and a third unstable radical of undetermined structure (31). The cation radical exhibited hyperfine couplings with one proton and two ^{14}N nuclei, consistent with significant delocalisation of the unpaired electron over C5, N1, and N3 atoms. The anionic radical displayed α-proton couplings indicative of a neutral radical isoelectronic with the isocytosine anion, while all radicals were highly unstable, disappearing at temperatures well below room temperature or upon irradiation with green Argon laser light. Together, these studies highlighted the sensitivity of minority and ionic radicals to both the molecular environment and the presence of impurities, providing deeper insight into the diversity of radiation-induced species in nucleic acid models and pyrimidine systems.

Following previous EPR studies on pyrimidine nucleotides, Herak and Rakvin investigated stable radicals in gamma-irradiated single crystals of deoxyguanosine 5′-phosphate (5′-dGMP, sodium salt) (32). At room temperature, three principal radical species were identified. The predominant radical resulted from hydrogen addition to C8 of the guanine ring, exhibiting EPR parameters consistent with N7-protonated H-addition radicals previously described in guanine hydrochloride. In addition, two other radical types were observed: a sugar-centred radical arising from furanose ring cleavage, and a base-associated radical, possibly a deprotonated cation or protonated anion within the guanine moiety. These findings complement earlier work on pyrimidine nucleotides such as 5′-UMP, where six radical species were identified, mainly formed *via* hydrogen or hydroxyl addition at the uracil ring (C5, C6, O2, O4). Notably, radicals in the uracil ring displayed greater stability when planar (O2 and O4 additions) compared to those involving C5 or C6. In contrast, the purine nucleotide 5′-dGMP showed a more complex radical behaviour, with the sugar moiety undergoing significant rearrangement after irradiation, resulting in allyl-type radicals with delocalized spin density over C4′, C5′, and O4′ (33). Herak and Rakvin further demonstrated that the radical formation pathways in purines can differ substantially from those in pyrimidines, likely due to differences in electronic structure and crystal packing, highlighting the diversity of radiation-induced damage in nucleic acids. Overall, the studies of Herak and Rakvin underscore the importance of both base and sugar contributions to radical formation, providing a detailed mechanistic framework for understanding the EPR signatures of irradiated nucleotides and the structural factors governing radical stability.

FACULTY OF PHARMACY AND BIOCHEMISTRY (1981–2006): EPR STUDIES ON SLOW OXIDATION OF LIPOPROTEINS

In addition to his investigations of free radicals in model systems of nucleic acid bases and their analogues, a second major focus of Herak's research concerned the slow oxidation of lipoproteins, as studied by EPR spectroscopy. These studies commenced in the early 1980s and continued uninterruptedly until the conclusion of his scientific career in the early 2000s. Building on his expertise in radical chemistry, Herak applied EPR spectroscopy to study lipoprotein oxidation, elucidating how structure, composition, and antioxidants influence transient radical formation and propagation, thereby advancing the molecular understanding of lipid peroxidation and oxidative stress in biological systems. This line of research also formed the basis for the doctoral dissertation of Nataša Stojanović and the M.Sc. thesis of Tatjana Ukrainczyk, reflecting the broader scientific impact of these investigations and their role in the training of a new generation of researchers in the field.

Prof. Herak's early investigations and application of EPR to lipoprotein systems extended to a detailed examination of molecular motion and organisation within human serum low-density lipoprotein (LDL). Employing proton magnetic relaxation across a temperature range of –30 to 30 °C, his first study identified two distinct mobile phases in LDL suspended in deuterated Tris-HCl buffer: a slow-relaxing phase, attributed to incompletely deuterated buffer water, and a fast-relaxing phase, corresponding to the fatty acid chains within the lipoprotein core (34). These observations revealed a pronounced correlation between the outer surface dynamics and the interior lipid environment, as the number of fast-relaxing protons was markedly influenced both by cooling through the buffer's freezing point and by variations in buffer ionic strength. At ambient temperature, approximately 30 % of lipid protons exhibited rapid relaxation on the NMR timescale (24 MHz), providing early evidence of the intricate coupling between lipid mobility and macromolecular structure within LDL particles.

Herak's investigations extended the use of Mn(II) ions as paramagnetic probes to characterise the ion-binding properties of lipoproteins. In human LDL, EPR measurements of unbound Mn(II) allowed quantification of the fraction of ions bound, revealing at least two distinct classes of binding sites: strong sites, few but high affinity, and abundant weak sites of lower affinity (35). The strong sites exhibited cooperative binding at low Mn(II) concentrations, while monovalent buffer cations could effectively block these sites at higher ionic strength, leaving the weak sites largely unaffected. Extending this approach to porcine LDL1, LDL2, and HDL, Herak and collaborators confirmed the generality of two binding site classes across lipoproteins, with weak sites showing similar affinities but varying in number (n = 114–135 for LDL, n = 28 for HDL), and strong sites of intermediate affinity with site numbers specific to lipoprotein type (n = 5–20) (36). Altogether, these studies suggested that the binding sites are primarily located in the protein components of lipoproteins and are largely associated with negatively charged amino acid residues, highlighting a conserved structural and functional organization in lipoprotein surfaces.

Several years later, Herak's initial investigations and application of EPR spectroscopy to lipoprotein systems were extended to the direct detection of free radicals in human low-density lipoprotein (LDL) using the spin-trapping approach (37). In collaboration with his colleagues, it was demonstrated that LDL solutions saturated with oxygen spontaneously generate and stabilise two distinct radical species within the lipid domain of the

particle. Although the precise nature of these radicals remained incompletely defined, kinetic and spectroscopic analyses suggested their assignment to lipid alkyl-type radicals (L·or LO·). These primary radicals initiate lipid peroxidation, a process that occurs even in the absence of exogenous antioxidants, ultimately leading to secondary modifications of the apoprotein and alterations in the functional properties of LDL. Herak and collaborators thus provided the first direct EPR evidence of free radical involvement in LDL oxidation, confirming the central role of lipid-domain radical formation in oxidative modification and underscoring the mechanistic basis for peroxidation-induced biological effects.

In subsequent investigations of LDL oxidation, following the publication of earlier results as a brief communication, Herak and collaborators examined the oxidation-induced increase in the net negative charge of low-density lipoproteins (LDL) using electrophoretic mobility measurements in conjunction with EPR spectroscopy (38). The observed increase in negative charge was attributed not only to the neutralisation of lysine residues within apoprotein B but also to the exposure of additional negatively charged residues on the LDL surface. This accumulation of negative charges was proposed to arise from conformational changes in apoprotein B, initiated by lysine neutralisation and peptide-bond cleavage. Alternatively, reactive oxygen species could mediate the conversion of specific amino acids, such as histidine to aspartic acid and proline to glutamic acid. Building on these findings, Herak formulated and published his *Oxidation Hypothesis of Atherogenesis* (39). This work provided compelling evidence that plasma lipoproteins, particularly LDL, can undergo *in vivo* modifications, most likely *via* oxidative processes, which in turn modulate their atherogenic potential. The study delineated the physicochemical changes induced by LDL modification, described the arterial microenvironment in which oxidation occurs, and outlined the subsequent metabolic fate of oxidised LDL. Special attention was given to the initiation of the oxidative process, highlighting, for the first time, that in addition to classical reactive oxygen species, alternative agents present within the LDL particle, influenced by diet or environmental factors, may serve as initiators of lipid oxidation.

Several years later, Herak and colleagues extended their investigations into the slow, spontaneous oxidation of low-density lipoprotein (LDL), focusing particularly on the dynamics of tocopherol-mediated peroxidation (TMP) (40). Using buffered LDL solutions supplemented with EDTA to chelate trace transition-metal ions, they observed that LDL undergoes slow autoxidation at physiological temperature, proceeding through three distinct phases. During the initial lag phase, no measurable changes in oxygen content, lipid hydroperoxides, or tocopherol levels were detected. This phase was interpreted as a period in which alpha-tocopherol is effectively protected or regenerated, likely by ubiquinol-10 or other lipid-soluble co-antioxidants, thereby preventing premature radical accumulation. In the subsequent propagation phase, oxygen consumption and formation of lipid hydroperoxides were observed, accompanied by the reversible cycling of alpha-tocopherol between oxidised and reduced states (41). This oscillation maintains the oxidation process active without net depletion of tocopherol. Free radicals, predominantly lipid-centred, were trapped primarily on the apoprotein, demonstrating that lipid-derived radicals rapidly interact with the protein moiety, effectively terminating radical diffusion. In the final phase, following near-complete oxygen depletion, the concentration of hydroperoxides decreased, while apoprotein-associated radicals persisted, confirming that the oxidation process culminates in the stabilisation of radicals on the protein. Mechanistically, the slow initiation of oxidation is postulated to arise from rare, chelated metal ions that catalyse

Fenton-like reactions, which are subsequently coupled to tocopherol-mediated chain-transfer reactions. The observed preservation of alpha-tocopherol throughout all phases of oxidation, despite ongoing radical formation, underscores its role as a dynamic mediator rather than a consumable antioxidant under conditions of very slow LDL oxidation. These studies not only clarified the kinetics of LDL autoxidation in the absence of exogenous pro-oxidants but also provided a molecular basis for the interaction between lipid peroxidation and apoprotein modification, highlighting the potential relevance of TMP *in vivo* for atherogenic processes.

In the final phase of his investigations into the slow oxidation of low-density lipoprotein (LDL), Herak formulated a probabilistic kinetic model of slow LDL oxidation, providing a theoretical framework capable of explaining the unusual kinetics observed in experimentally studied systems (42). The model was developed to account for the very slow autoxidation of LDL under conditions deliberately designed to minimise oxidative initiation, where only trace amounts of transition-metal ions associated with the lipoprotein particles are assumed to trigger the process. In this framework, the primary substrates for metal-ion attack are alpha-tocopherol and pre-existing lipid hydroperoxides. The model assumes oscillatory cycling of both the transition-metal ions and alpha-tocopherol between their oxidised and reduced states. Under such conditions, alpha-tocopherol can function as a pro-oxidant mediator, participating in tocopherol-mediated peroxidation (TMP). A central conceptual feature of the model is that LDL oxidation does not proceed uniformly throughout the system but rather occurs as rare, stochastic bursts of radical reactions within individual LDL particles. The microscopic reaction network inside each particle is therefore described in terms of probabilities for specific reactive species to participate in reactions. The cyclic propagation of radical reactions can thus be interpreted as a circular flow of microscopic probabilities. Within this probabilistic framework, experimentally measurable macroscopic quantities, such as the rates of oxygen consumption, hydroperoxide formation and decay, depletion of co-antioxidants, and accumulation of trapped radicals, are quantitatively linked to the underlying microscopic probabilities. The kinetics of the system are described by a set of differential equations representing the generation and propagation of radicals, which were solved numerically using a finite-difference approach and parameterised by a small number of adjustable kinetic constants. Application of the model to experimental measurements of multiple oxidation markers during slow LDL oxidation demonstrated that the process can be consistently described as tocopherol-mediated peroxidation sustained by trace copper ions present in LDL particles (43). In this mechanism, both copper ions and alpha-tocopherol act as catalytic components that oscillate between oxidised and reduced states, allowing the oxidation process to proceed while the overall concentration of alpha-tocopherol remains essentially unchanged. Fitting the theoretical model to experimental data obtained from LDL samples derived from multiple donors yielded remarkably consistent values of the kinetic parameters. By introducing this probabilistic kinetic description, Herak provided one of the earliest quantitative theoretical treatments of LDL oxidation at the single-particle level, bridging experimental EPR observations with a microscopic mechanistic model of radical processes occurring within lipoprotein particles.

Finally, Herak concluded his investigations on the slow oxidation of high-density lipoproteins (HDL) with an EPR study in which he examined the oxidation kinetics of the two major HDL subclasses, HDL_2 and HDL_3, under conditions favouring extremely slow,

metal-ion-mediated autoxidation (44). Extending the experimental strategy previously developed for LDL systems, the oxidation process was monitored by measuring the time dependence of oxygen consumption and the accumulation of spin-trapped free radicals using EPR spectroscopy. The results revealed that the dependence of the oxidation process on the copper-to-lipoprotein molar ratio differs markedly from that observed in LDL dispersions, indicating distinct mechanistic features of oxidative processes in HDL particles. Comparison of the kinetic profiles of HDL_2 and HDL_3 demonstrated that, under all examined experimental conditions, HDL_2 exhibits a higher susceptibility to copper-induced oxidation than HDL_3. These findings provided further insight into the physicochemical determinants governing oxidative transformations of plasma lipoproteins and suggested that oxidative modification may influence the atheroprotective functions traditionally attributed to HDL. In this way, Herak's work extended the conceptual framework developed for LDL oxidation to HDL systems, offering a broader perspective on the role of slow, metal-ion-mediated oxidative processes in lipoprotein biology.

FACULTY OF PHARMACY AND BIOCHEMISTRY (1991–2003): EPR STUDIES ON LONG-RANGE ENERGY TRANSFER IN NUCLEIC ACID BASES AND ANALOGUES

In parallel with his investigations of LDL oxidation, Herak continued his research on free radicals in model systems of DNA bases and their analogues using EPR spectroscopy. Within this line of research, he initiated systematic studies of long-range energy transfer processes occurring along these molecular model systems, employing radical intermediates as sensitive spectroscopic probes of electronic interactions and energy migration within nucleic acid-related structures. These investigations also led to the doctoral dissertations of Krešimir Sanković and Tatjana Pranjić-Petrović. The work of Krešimir Sanković further developed the experimental and theoretical framework for studying long-range energy transfer processes in nucleic acid base systems using EPR spectroscopy.

The pioneering work on long-range energy transfer processes in nucleic acid base systems using EPR spectroscopy focused on model crystalline systems of nucleic acid bases containing trace amounts of their sulfur analogues as structural impurities (45). Polycrystalline cytosine- and guanine-based crystals doped with small amounts of thiocytosine or thioguanine, respectively, were X-irradiated at 77 K, resulting in strong EPR signals characteristic of sulfur-centred radicals influenced by the surrounding base matrix. In contrast, thymine systems, non-matching base-thiol combinations, and samples prepared as frozen glasses, aqueous solutions, or most freeze-dried mixtures did not exhibit these radicals. Taken together, these observations indicate that the crystalline organisation of nucleic acid bases supports long-range migration of positive charge (holes), providing early experimental evidence that structural arrangement within base assemblies facilitates long-range electronic interactions relevant to radiation-induced processes in DNA.

Following this study, the work by Herak and his colleagues on postirradiation long-range energy transfer in single crystals of cytosine monohydrate demonstrated that thiocytosine molecules incorporated into the crystal lattice act as traps for both electrons and holes (46). Radiation-induced cytosine ion radicals of cationic and anionic type release their charge upon heating, allowing excess electrons and holes to migrate over long distances. EPR spectroscopy revealed the thermally activated accumulation of thiocytosine

cationic radicals, indicative of hole migration, while formation of sulfur-centred anionic radicals indicated electron migration. The estimated migration lengths were at least 30 base-to-base distances for holes and more than 100 base-to-base distances for electrons. These observations highlighted the selective formation of cationic and anionic trap radicals depending on trap concentration and established that the long-range migration of electrons and holes in ordered base stacks is a postirradiation, thermally activated process, mediated by the crystal lattice and stabilised by thiol impurities.

Building on this work, single crystals of cytosine hydrochloride containing thiocytosine as an impurity were examined to explore a potential new mechanism of long-range energy migration induced by ionising radiation (47). In these crystals, two types of chlorine-containing paramagnetic centres were observed with thiocytosine molecules and Cl^- ions acting as hole traps. EPR characterisation indicated that one centre derives from the cationic thiocytosine radical interacting with a Cl^- ion, while the other results from $Cl\cdot$ interacting with a thiocytosine molecule, suggesting that the migration of electron-loss sites (holes) through the Cl^- network underlies long-range energy transfer. Advancing this line of research, two different crystals of 5-methylcytosine doped with 5-methylthiocytosine were studied under irradiation (48). In 5-methylcytosine hemihydrate, two thiyl-type radicals were observed, with the unpaired electron primarily on sulfur, whereas doped 5-methylcytosine hydrochloride exhibited distinct paramagnetic species with high spin density on chlorine, one of which involved delocalisation between sulfur, chlorine, and the pyrimidine ring. Both neutral and positively charged 5-methylcytosine proved effective as radiation energy traps, confirming the generality of long-range energy and charge migration in ordered nucleic acid base assemblies.

Subsequent investigations were directed toward guanine (49). Single crystals of guanine hydrochloride monohydrate (G(TG)M), dihydrate (G(TG)D), and anhydrous guanine dihydrochloride (G(TG)A), doped with trace amounts of thioguanine, were subjected to X- and gamma-irradiation to elucidate the mechanisms of hole transfer and radical stabilisation. EPR analysis of G(TG)M and G(TG)D demonstrated the formation of thiyl radicals at the thioguanine sites, arising from electron loss at guanine, followed by migration of the resulting hole and subsequent deprotonation. The organisation of stacked guanine bases in conjunction with one-dimensional arrays of chloride ions facilitated long-range hole migration. In contrast, G(TG)A exhibited chlorinated thioguanine-centred radicals. In this system, neither the guanine bases nor the chloride ions alone sufficed to support long--range charge migration; rather, an alternating network of guanine cations and chloride anions provided the necessary conduits. The occurrence of chlorination was dictated by the local availability of chloride ions and the steric accommodation around the sulfur atom. Collectively, these observations indicate that all three guanine hydrochloride lattices support long-range hole migration, with thioguanine impurities acting as highly efficient radical traps. The extent and nature of radical stabilisation were governed by the immediate structural and ionic environment, extending the mechanistic insights previously established for cytosine-based systems.

Two subsequent electron nuclear double resonance (ENDOR) studies further elucidated the structural and electronic characteristics of sulfur-centred radicals in nucleobase crystals. In the first study, ENDOR spectroscopy was applied to X-irradiated single crystals of cytosine monohydrate, substitutionally doped with 2-thiocytosine, at 15 K (50). The electron-loss radicals were highly enriched at the thiocytosine sites, exhibiting a concen-

tration enhancement factor exceeding 45 in comparison to the nominal thiocytosine/cytosine ratio. The radicals were readily distinguished in EPR spectra by their elevated g-values. Six distinct intra- and intermolecular proton couplings were resolved, enabling the assignment of couplings to protons within the N1-deprotonated thiocytosine radical and its surrounding lattice. The spin density was predominantly localised on sulfur (≈ 65–70 %), with minor contributions at C5 and N1, contrasting with the distribution in the corresponding cytosine radical. The observed enrichment indicates long-range hole migration, with an estimated transfer range exceeding 22 base separations (≈ 7 nm). The second study investigated the chlorinated thiocytosine radical, containing an S∴Cl three-electron bond, embedded in the cytosine hydrochloride lattice (51). ENDOR analysis revealed interactions with at least seven protons, five of which were characterised. Three couplings originated from protons of the S∴Cl radical itself, while two arose from neighbouring cytosine molecules. Hyperfine tensor analysis indicated that the unpaired electron is partially delocalized: concentrated in the C2–S–Cl region but with significant spin density extending over the cytosine π^* system. Computational DFT studies corroborated this spin distribution, which retains protonation at both N1 and N3 positions. These two studies collectively demonstrate the influence of both lattice environment and chemical modification on radical localisation, spin density distribution, and long-range hole migration in nucleobase crystals.

Prof. Herak concluded his investigations into long-range hole transfer in nucleobase crystals with a study of gamma-irradiated 2-thiothymine (52). This system was shown to uniquely generate a σ-type radical, in contrast to the π-type radicals observed in all other nucleic acid bases and thionucleobases studied so far. The formation of this radical, resulting from the deprotonation of the cationic precursor, highlights a distinct electronic symmetry specific to 2-thiothymine. Its discovery not only expands the understanding of radiation-induced radical formation in ordered nucleobase lattices but also provides a rare example of a σ-centered radical in a naturally derived heterocyclic system, underscoring the compound's exceptional photochemical and radiation-responsive properties.

OTHER RESEARCH ACTIVITIES

Thermal decomposition and reaction sites of barbituric acid derivatives

In 1972, Herak shortly extended his studies to the thermal decomposition and reaction sites of barbituric acid derivatives, publishing two notable articles. The first article focused on identifying free radicals formed during heating of compounds such as alloxan, uramil, violuric acid, dilituric acid, and cyclobarbital using EPR spectroscopy (53). Herak demonstrated that different decomposition pathways (loss of substituents, modification of substituents, or ring scission) lead to distinct radical species, providing direct insight into intermediate products of thermal degradation. This work highlighted the potential of EPR spectroscopy to probe subtle molecular transformations, even in solid-state pharmacologically relevant compounds. The second article investigated the most reactive sites in barbituric acid derivatives upon exposure to thermal hydrogen atoms and excited argon atoms (54). The study revealed that the C5 substituent is the predominant site of reaction, with pharmacologically active derivatives showing changes in the substituent and inactive ones undergoing simple loss. Remarkably, the radicals generated by chemical and physical

energy were essentially identical, demonstrating a fundamental selectivity in radical formation that is largely independent of the type of energy applied. These findings contributed to understanding the relationship between molecular structure, reactivity, and pharmacological activity in barbituric acid derivatives.

Interaction of Cu(II) ions with nucleic acid bases and their analogues

Towards the later stages of his career, Herak also investigated the interactions of Cu(II) ions with nucleic acid bases and their synthetic analogues using EPR spectroscopy. Within this research, EPR studies allowed the precise identification of Cu(II) binding sites in single crystals of cytosine and 2-thiothymine. This line of inquiry not only deepened the understanding of metal–nucleobase interactions but also provided a foundation for further studies, including the doctoral dissertation of Erim Bešić, which focused on this subject.

The first two studies investigated the interaction of Cu(II) ions with cytosine molecules, focusing on how Cu(II) binds within the crystal matrix and alters the molecular environment (55, 56). Using EPR spectroscopy, the research revealed the formation of two distinct copper-cytosine complexes with different coordination geometries and magnetic properties. Complex I is initially diamagnetic in as-grown crystals and becomes paramagnetic upon ionising irradiation. The Cu(II) ion coordinates with three nitrogen atoms from two adjacent cytosine molecules, effectively replacing two hydrogen bonds present in the unmodified crystal. The paramagnetic centre forms through irradiation-induced oxidation of Cu(I) to Cu(II), acting as a sink for migrating holes in the crystal matrix. Complex II is paramagnetic in as-grown crystals, indicating that Cu(II) is incorporated directly during crystallisation. Here, the copper ion coordinates with one nitrogen atom from a cytosine amino group and three oxygen atoms, one from the opposing cytosine and two from water molecules. EPR and DFT analyses show that the copper ion sits between two ribbons of cytosine molecules, causing a concerted restructuring of the hydrogen-bond network, confirmed by Raman spectroscopy. These studies highlight that Cu(II) ions can adopt multiple coordination modes within cytosine crystals, with significant effects on the hydrogen-bonding framework and distinct spectroscopic signatures. The results also resolve ambiguities from earlier studies, providing a detailed understanding of metal--nucleobase interactions relevant to nucleic acid chemistry and materials science.

Another detailed EPR study explored the interaction of copper ions with 2-thiothymine molecules in single crystals containing traces of copper (57). Initially present as a Cu(I) complex in the crystal lattice, the copper impurities are transformed into paramagnetic Cu(II) centres upon ionising irradiation. The resulting complex is planar, with the copper coordinated by two sulfur and two nitrogen atoms from adjacent thiothymine molecules, effectively replacing a pair of hydrogen bonds with stronger coordination bonds. Angular-dependent measurements allowed the determination of the g- and A-tensors, showing excellent agreement with the crystallographic arrangement of the pyrimidine rings. This study demonstrates that Cu(II) can form stable, planar N2-S2 complexes with thionucleobases, replacing weaker hydrogen bonds and acting as efficient traps for irradiation-induced holes. The findings are consistent with earlier observations in cytosine crystals, suggesting that metal coordination can significantly influence the

hydrogen-bond network in nucleobase assemblies and potentially in DNA-like structures.

Continuing the line of investigation established in these studies and building upon the analytical principles applied therein, the analysis originally initiated by Herak has recently been completed and published (58). This subsequent work further examined the interaction of Cu(II) ions with nucleic acid bases in crystalline environments using EPR spectroscopy in combination with complementary theoretical approaches. By extending the methodological framework developed in the earlier investigations, the study provided additional insight into the coordination behaviour of copper ions and their influence on the hydrogen-bonding network and electronic structure of nucleobase assemblies. In doing so, it represents a continuation and completion of the research direction that Herak had initiated, confirming the broader relevance of metal–nucleobase interactions for understanding charge trapping, structural rearrangements, and radiation-induced processes in nucleic acid model systems.

Theoretical and computational investigations into electronic structure and charge trapping in nucleobase systems

At the end of his scientific career, Herak devoted himself to theoretical and computational investigations into the electronic structure, charge migration, and radical trapping in nucleic acid bases and their analogues. These studies, which also gave rise to the doctoral dissertation of his final student, Vjeran Gomzi, encompassed detailed quantum-chemical analyses of spin density distributions, ionisation potentials, and intermolecular interactions relevant to hole and electron trapping in ordered base assemblies.

In one of the seminal contributions, Herak and Gomzi employed density functional theory (DFT) at the B3LYP level to model the spin density distribution and proton hyperfine coupling tensors of the neutral thiocytosine electron-loss radical in the crystal matrix of cytosine monohydrate (59). The study demonstrated that isolated radical models fail to reproduce key experimental ENDOR features, whereas cluster models incorporating one or more neighbouring cytosine molecules yield spin distributions and proton coupling tensors in close agreement with observations. Notably, these calculations confirmed intermolecular proton transfer along hydrogen bonds, specifically from the thiocytosine H1 position to the N3 site of an adjacent cytosine molecule, representing the first complete DFT analysis of distant proton coupling tensors for a radical in a solid environment.

Complementary investigations addressed charge trapping and ionisation potentials (IPs) of nucleic acid bases and their thioanalogues using the partial third-order electron propagator (P3) method (60). These studies elucidated the electronic basis for selective hole trapping, revealing that thioanalogs consistently exhibited lower IPs than the parent bases, rationalising their empirical effectiveness as hole traps in stacked base systems. Among the molecules studied, mercaptoguanines showed the lowest IPs, whereas comparisons between P3 and Koopmans approaches indicated that different computational schemes could yield distinct energy level orderings, highlighting the importance of methodological rigour in theoretical analyses of charge migration.

Further refinement of the theoretical modeling was achieved through g-tensor calculations for thiocytosine radicals in crystal matrices using B3LYP/6-311G(2d,p) for both geometry optimisation and single-point calculations (61). These studies demonstrated that

the theoretically derived g-tensor principal values and direction cosines align well with experimental measurements when the spin density distribution over neighbouring molecules is incorporated. Inclusion of more molecules in the computational cluster improved agreement with experiment, emphasising the need for careful lattice truncation and partial structural optimisation to account for local rearrangements near the radical site.

In summary, these theoretical and computational studies significantly advanced the mechanistic understanding of charge distribution, radical stabilisation, and electronic structure in nucleobase systems, bridging experimental EPR/ENDOR observations with detailed quantum-chemical insights and reinforcing the conceptual framework for long--range energy migration and trapping in ordered biological and model materials.

CONCLUSIONS

The scientific career of Janko N. Herak represents one of the most influential contributions to the development of electron paramagnetic resonance (EPR) spectroscopy and free-radical research in Croatia and the broader scientific community. Over more than four decades, his work progressed from fundamental spectroscopic investigations of small molecules and radiation-induced radicals to increasingly complex biological systems, including nucleic acids and plasma lipoproteins.

Herak's early research established a rigorous experimental framework for the identification and structural characterisation of radiation-induced radicals in nucleic acid bases and their derivatives. Through systematic EPR and ENDOR studies of single crystals, his work provided detailed insights into hydrogen-addition and hydrogen-abstraction mechanisms, radical stability, and the influence of molecular and crystal structure on radical formation. These studies contributed significantly to the understanding of radiation damage in biologically relevant molecules and established nucleic acid model systems as valuable platforms for investigating radical chemistry.

In later decades, Herak expanded his research to the study of oxidative processes in biological macromolecular systems, particularly the slow oxidation of lipoproteins. By combining EPR spectroscopy with kinetic modelling, he elucidated mechanisms of lipid peroxidation and radical propagation in LDL and HDL particles and proposed theoretical frameworks describing the probabilistic kinetics of slow lipoprotein oxidation. These studies provided important insights into oxidative modifications of lipoproteins and their potential relevance to atherogenic processes.

Parallel to these investigations, Herak continued exploring fundamental aspects of radical chemistry in nucleobase assemblies, including long-range charge migration and energy transfer in ordered crystalline systems. These studies demonstrate that nucleic acid base stacks can support long-distance migration of electrons and holes, with sulfur analogues acting as efficient radical traps. His later work further integrated experimental spectroscopy with quantum-chemical modelling to explain radical stabilisation and electronic structure in nucleobase systems.

Taken together, Herak's research illustrates a coherent and evolving scientific trajectory, characterised by methodological precision, interdisciplinary breadth, and a sustained focus on the molecular mechanisms of radical formation and transformation. Beyond his numerous scientific publications, his legacy is reflected in the establishment of EPR spec-

troscopy in Croatia, the development of experimental infrastructure, and the mentoring of several generations of researchers. His work continues to influence contemporary studies of radiation chemistry, radical processes in biological systems, and EPR spectroscopy.

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