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The History of Determination of Bio-Antioxidants and Neuromediators by GC- and GC-MS Techniques (Translational Lecture Course Summary)

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ABSTRACT

This introductory review examines the historical and methodological aspects and prospects for the application of gas chromatography in redox biology and neurochemistry. In particular, the following topics are covered: the history of gas chromatography and GC-MS of thiols; the history of gas chromatography and GC-MS of acetylcysteine; the history of gas chromatography and GC-MS of acetylcholine; the history of gas chromatography and GC-MS of catecholamines and their metabolites; the history of gas chromatography and GC-MS of hydroperoxides; and the history of gas chromatography and GC-MS of phosphatidylcholine.

Keywords: Gas chromatography, Thiols, Acetylcysteine, Acetylcholine, Catecholamines, Hydroperoxides, Phosphatidylcholine, GC-MS, Antioxydants, Bio-antioxydants, Redox-etiology, Redox-pathology, Neuromediators, Neurotransmitters

1. The Practical Importance of Analytical Determination of Antioxidants

This article draws on several previously unpublished bibliographic compilations and seminar materials prepared over roughly a decade. The core foundation consists of annotated working lists on chromatographic methods for neurotransmitters, antioxidant chemistry and neurochemical applications of gas chromatography and GC–MS, assembled at different stages of the second author's academic and laboratory work (from pre-graduation research through 2016) and later expanded in the early 2020s with contributions from all co-authors. Each set of documents provided a defined share of the present bibliography and conceptual outline, with the largest inputs coming from neurotransmitter-focused chromatography and GC–MS surveys accumulated during dissertation-related work and subsequent employment in research laboratories.

The preparation history also reflects the institutional and technical constraints under which the material was collected. Multiple research initiatives were interrupted by contract termination, laboratory reorganization, dissolution of units following broader institutional reforms and progressive obsolescence and loss of GC/GC–MS instrumentation and facilities. An updated integrative bibliographic dossier produced for internal planning in the early 2020s ultimately remained unused due to further relocations and degradation of laboratory infrastructure. Given the accelerating loss of technical capacity and the improbability of converting the accumulated bibliographic groundwork into new experimental GC/GC-MS studies, the authors decided to publish the present abridged review version as the most feasible outcome under current conditions, without attempting to expand it into a full-scale high-impact review.

A major practical challenge associated with the medical use of various antioxidants[1] is their detection in natural samples[2,3], pharmaceutical substances and their precursors[4,5], as well as in human tissues and physiological excreta. Without documenting the redox status of the latter, the transition from redox biology[6-9] to redox medicine[10-14] cannot be meaningfully advanced. This implies that, in the absence of objective assessment of redox status, a constructive approach to redox pathology[15-20], redox aetiology[21,22] and redox-based disease prevention[23,24]-achieved through dietary intake of antioxidant-containing foods and supplements[25-37]-is likewise unattainable.

Clearly, if agents acting as antioxidants in metabolism cannot be analytically monitored, their tissue levels cannot be regulated, nor-ideally-can the antioxidant-induced status of cells and tissues be controlled, at least with the aim of reaching a range of optimal concentrations associated with predictable physiological effects[38-40]. Therefore, technologies in the analytical chemistry of antioxidants[41-45] and, in particular, in the analytical biochemistry of antioxidants[46-48] are of critical importance for formulating problems in redox medicine. At the same time, many methods used for these purposes quantify activity rather than specific chemical systems that are selective with respect to composition and reactivity in a given medium. Examples include potentiometric determination of redox-active species (in particular radical species[49]) and antioxidants[50-54].

If one accepts that many antioxidant schemes operate efficiently in microheterogeneous/ultramicroheterogeneous media[55] and that biological tissue containing organelles with different electrophysical parameters represents a highly characteristic example of such media, it becomes evident that additive analysis, as well as evaluation based on overall redox efficiency, is unfortunately insufficiently rigorous for the purposes of redox medicine. At a minimum, the concentrations of different redox components and antioxidants must be assessed separately; at a maximum, the analysis should be position-sensitive-performed within the compartments in which the reaction processes occur-regardless of whether plant or animal tissues are considered[56-65].

The second task pertains to classical cytochemistry or ultrastructural biochemistry and is therefore not considered here, whereas the first task is relatively straightforward from the standpoint of point measurements or sample-averaged determinations-i.e., it can be addressed using standard methods of bioanalytical chemistry[66-72]. In particular, since the early 2000s, gas-chromatographic methods for antioxidants[73-87]-most actively developed in the context of GC–MS, against which other gas-chromatographic techniques “fade, losing their classical luster”[88,89]-may be regarded as an optimal means of achieving such analytical objectives. Nevertheless, classical gas-chromatographic techniques can also be employed for these purposes, as will be shown below.

2. On “dialectical mechanisms” in Redox Physiology

At the same time, even under this simplified approach, the chemical physics of molecular–biological regulatory systems remain unchanged, constituting a dialectical[90-99] reflection of the mechanisms underlying the corresponding physiological and biochemical reactions. For example, for the well-known thiols, pro-oxidant and antioxidant effects coexist[100-109]. The recently cited study[108] demonstrates that the pro-oxidant effects of native thiols are associated with reactions of thiyl radicals, which readily add to unsaturated bonds; these radicals are formed, in particular, in thiol exchange reactions with other radicals and during thiol interactions with hydroperoxides.

At the same time, in solution at physiological temperature, the antioxidant mechanisms of endogenous thiols (glutathione, cysteine, homocysteine) include reactions with reactive oxygen species-peroxyl radicals (and hydrogen peroxide); notably, reduction of the latter (hydrogen peroxide) is accompanied by radical formation. The phenolic antioxidants used in the aforementioned work (resveratrol and caffeic acid) are consumed upon interaction with glutathione and the reaction is accelerated in the presence of hydrogen peroxide. Thus, thiols provide an example that illustrates the full range of antagonistic and competitive, as well as cooperative and synergistic, processes characteristic of the chemistry of such systems-excluding those determined by additional phases and compartmentalizing boundaries. For solution chemistry and the chemistry of homogeneous media (both aqueous and non-aqueous[110,111]), this is sufficient.

3. Excursus into the Chemistry of Microheterogeneous Systems and Membrane Mimetics

For the chemistry of microheterogeneous systems, the situation appears even more complex[112-117], both in terms of solute behavior in micelles and in terms of photometric and spectrophotometric determination, as well as chromatography and chromatography–mass spectrometry of such systems-procedures that become almost impossible without disrupting the microheterogeneous structure. In other words, the method ceases, to any meaningful extent, to qualify as non-destructive, owing to the emergent properties of microheterogeneous assemblies and the irreducibility of those properties to the behavior of the individual components.

In our work, the principal practical sources concerning the behavior of the microheterogeneous systems of interest are publications from the Laboratory of Liquid-Phase Oxidation of the N.N. Semenov Institute of Chemical Physics, Russian Academy of Sciences, which effectively continues the research tradition in homolytic reactions established by Academician N.M. Emanuel and his colleagues. This laboratory provides a bridge to several aspects that are largely beyond the scope of the present review but are important for understanding the neurochemical dimension of phenomena involving neurotransmitters/neuromediators, in particular:

- The influence of membrane lipids on free-radical generation in acetylcholine-containing systems[118]
- The combined (superadditive, microstructure-dependent) contributions of lipids/surfactants and antioxidants in similar microheterogeneous systems[119,120];
- The interactions of thiols and catecholamines with reactive oxygen species[121];
- Catalysis of radical reactions in mixed surfactant micelles in the presence of hydroperoxides.

- This line of research has a broader physicochemical context. In[122], the authors note that:
    - In oxidizing hydrocarbons (RH), hydroperoxides (ROOH)-the primary amphiphilic oxidation products—form mixed micelles with cationic surfactants, {m\mathrmROOH··· n\mathrmCS$^+$}, in which ROOH decomposition into radicals is accelerated and in which other polar components (metal compounds, inhibitors, etc.) may become concentrated; this substantially affects both the rate and the mechanism of oxidation. It is shown that cationic surfactants immobilized on a solid support retain the ability to catalyze hydroperoxide decomposition with radical formation and to initiate radical oxidation and polymerization processes.
    - The specific features of the catalytic action of cationic surfactants, in combination with hydroperoxides, on radical generation are considered, as is the influence of various factors on this process, including transition-metal compounds, oxygen and an external magnetic field.
- These considerations are applicable to neurotransmitter-containing systems, including, specifically, acetylcholine-containing systems. Thus,[119,120] reports that:
- Cationic surfactants (S^+) and acetylcholine (ACh; a major neurotransmitter that plays a substantial role in the neuromuscular and cognitive activity of living organisms) form mixed reverse micelles with hydroperoxides (ROOH) in organic media, in which the catalytic decomposition of ROOH into free radicals is accelerated.
- The addition of cholesterol (Chol; 30 mol%) to pyridinium bromides (CPB) and cetyltrimethylammonium bromide (CTAB) reduces, by several-fold, the rate of radical generation during the catalytic decomposition of ROOH.
- …in the case of less ordered and larger reverse micelles ACh–ROOH, an increase in the rate of radical initiation is observed.
- The addition of Chol has virtually no effect on the micelle sizes of CTAB and CPB with hydroperoxides, but leads to a decrease in micelle size in the case of ACh–ROOH.

On the one hand, a "bridge" between the chemistry of such microheterogeneous systems and neurochemistry is readily apparent, because in nervous tissue acetylcholine (ACh) is also conveyed in the form of microheterogeneous entities - synaptosomes. Although synaptosomes are not fully equivalent to the micelles used in model microheterogeneous systems, they nevertheless represent prototypical objects for model descriptions of ACh-signal transmission[123-143].

On the other hand, emphasizing the role of cholesterol naturally connects this line of inquiry with nutraceutical science and foodomics, while cholinergic neuromuscular synapses, in this context, additionally link ACh-containing microheterogeneous systems to myology. One may also raise the question of whether electrophysiological and magnetobiological phenomena in ACh neurotransmission are related to the structure of microheterogeneous "neuromimetic" micellar systems that carry those "quanta" of the ACh neurotransmitter and that can likewise be transmitted-at least in model systems. Thus, Kasaikina and Pisarenko report the following:

- Oxygen and an external magnetic field were found to exert a retarding effect on the rate of radical initiation during hydroperoxide decomposition in catalytic nanoreactors. Mixed reverse micelles formed by a cationic surfactant and a hydroperoxide, {mLOOH...n surfactant}, served as the nanoreactors.
- Similar effects of oxygen and an external magnetic field (60-150 mT) on the rate of radical initiation were observed for the catalytic radical decomposition of a hydroperoxide in the presence of acetylcholine.
- Notably, the retarding effect of the magnetic field decreased in the presence of paramagnetic species—oxygen and relatively stable radicals.

Similarly, Kasaikina, et al. state[144]:

- It was established that acetylcholine, a key neurotransmitter that plays a substantial role in neuromuscular and cognitive activity in living organisms, in organic media catalyzes the radical decomposition of hydroperoxides in a manner analogous to cationic surfactants; under these conditions, the radical yield decreases in a magnetic field and in the presence of oxygen.

Accordingly, one can substantiate, on the one hand, the need to include acetylcholine and other neurotransmitters in the discussion as antioxidants and, on the other hand, the need to consider thiols.

4. History of Gas Chromatography and GC–MS of Thiols

To begin, it is useful to consider-using a concrete example-methodological approaches to the additive separation and practical identification of specific redox factors and antioxidants that are applicable (or relevant) to physiology and medicine. In view of the above, thiols appear to be a reasonable starting point. Given the reproducibility of a range of analytical methods for thiols, similarly to other redox agents and antioxidants, it was decided to implement this approach on the basis of gas chromatography.

The earliest studies on the gas chromatographic analysis of thiols-particularly on their separation from other components in complex mixtures-date to the early 1960s[145]. For a long time, however, widespread adoption was constrained by the technical capabilities available at the time, which were insufficient for efficient analysis. The introduction in the 1980s of methods that simplified thiol analysis (including headspace gas chromatography[146]) enabled a marked intensification of research in the 1990s. In particular, the emergence of affordable MS detectors and integrated GC-MS hardware–software platforms made it possible to develop experimental protocols that included not only chromatographic separation but also direct identification, including multiparametric identification using multiple detectors, in native and synthetic matrices containing thiols[147-149]. It also became feasible to compare data obtained by methods using different phases-for example, GC–MS and HPLC.

By the mid-2000s, the predominance of GC–MS over "stand-alone" GC in thiol analysis had become established and this trend began to extend to catalytically generating systems employing various agents, including photocatalysts and others[150]. Subsequent microminiaturization enabled analyses not only on conventional columns but also on capillary or needle-type columns, including chromatographic–mass-spectrometric formats such as NTD-GC–MS[151].

In the mid-2010s, applications of tandem GC–MS to these tasks became increasingly prominent[152,153]. Improved mass-spectrometer resolving power-including that of designs integrally coupled to GC–MS systems and used in MS/MS-enabled precise and reproducible isotope analysis[154]. In addition, the advent of field-deployable and mobile mass spectrometers made it possible to integrate gas chromatographic analysis with olfactometry (as an alternative to "electronic nose" technologies) for comparative odorological and flavor-chemical studies. During the same period, gas chromatographic methods that do not rely on mass-spectrometric detection also continued to develop, but substantially more slowly, as their capabilities have largely been exhausted. Nevertheless, notable advances include the chiral gas chromatographic separation of products of thiol-ene synthesis / "click chemistry"[155]. To date, most applied studies involving natural raw materials are generally performed using conventional GC-MS, albeit with various methodological innovations at the stages of sample preparation and separation[156].

Using an "inhibitor method," it has been established that the reduction of hydrogen peroxide by thiols in aqueous solutions is typically accompanied by radical formation. Specifically, the interaction of glutathione and the synthetic/semi-biosynthetic thiol N-acetylcysteine with hydrogen peroxide at \mathrmpH<7 generates thiyl and hydroxyl radicals[157]. This, in particular, supports the use of luminescence methods and electron paramagnetic resonance in addition to mass spectrometry.

5. History of Gas Chromatography and GC–MS of N-Acetylcysteine

As noted above, the interaction of the thiol N-acetylcysteine (NAC) with hydrogen peroxide in the presence of glutathione is of particular interest. Accordingly, it is reasonable to consider next the methodological development of GC and GC–MS approaches for NAC determination.

In this context, the following can be noted. GC analysis of NAC has been performed at least since the late 1970s[158]. By the early 1990s, these assays had largely transitioned to capillary GC formats[159], reflecting the general shift toward higher-efficiency separations and improved sensitivity.

In parallel, GC coupled with mass-spectrometric detection was introduced for NAC measurements, including applications in blood plasma and other biogenic, partially structured matrices[160]. By the 2000s, GC-MS-based determination of NAC (and related sulfur-containing metabolites) had been incorporated into clinical laboratory diagnostic workflows in both the United States and the European Union, for example in routine urine analysis[161]. As a result, the core analytical tasks of identification and clinical interpretation based on mass spectrometry-also encompassing NAC quantification for pharmacokinetic and pharmacodynamic purposes-are now well supported by established clinical MS practice.

6. History of Gas Chromatography and GC-MS of Acetylcholine

The gas-phase determination of neurotransmitters is of considerable interest, as it aligns well with the conceptual framework of "gas biology" and lays the groundwork for qualitatively new directions such as gas neurochemistry and gas-phase biochemical neurophysiology. One of the best-known neurotransmitters of the parasympathetic nervous system is acetylcholine. Purely gas-chromatographic methods for acetylcholine determination have been known since the 1960s[162-164]. As a rule, these studies employed pyrolytic gas chromatography[165-171]. However, from the second half of the 1960s onward, combined gas chromatography/mass spectrometry approaches also began to be introduced[172-177], including methods capable of measuring acetylcholine at subpicomolar amounts-although this did not preclude the continued use in the 1970s and later of conventional gas[178,179] or gas-liquid[180,181] chromatography.

The integration of pyrolytic gas chromatography and mass spectrometry in the analysis of acetylcholine and other neurotransmitters took place in the mid-1970s[182-184] and reached conceptual maturity in the 1980s, with the emergence of pyrolytic chromatography with MS detection, achieving at least picomolar concentration levels and being integrated with mass fragmentography[185-188]. In acetylcholine analysis, the era of pyrolytic chromatography ended in the 1990s for reasons related to the advent of more sensitive technologies[189].

By that time, mass-spectrometric analytics had already developed methods such as secondary-ion mass spectrometry for compounds of this type[190], surface-ionization mass spectrometry[191], MALDI[192] and liquid chromatography with mass-spectrometric detection-including tandem mass spectrometry[193] and micro- and semi-micro-HPLC inlet technologies[194]-as well as fast-atom bombardment methods[195,196]. In response to increased analytical requirements and improved sample-preparation strategies, GC-MS was combined with intracerebral microdialysis[197]. In GC-MS, an approach based on the interpretation of electron-impact mass spectra became established[198,199]. Nevertheless, a purely pyrolytic approach continued to be applied in the 1990s as well[200].

7. Historical development of gas chromatography and GC–MS for catecholamines and their metabolites

Catecholamines are generally defined as physiologically active compounds-derivatives of pyrocatechol-that act as chemical messengers and regulatory molecules (mediators and neurohormones) in intercellular communication in animals and humans, including within the brain. As is well known, this class includes neurotransmitters such as adrenaline/epinephrine, noradrenaline/norepinephrine and dopamine.

The history of gas-chromatographic determination of catecholamines and their metabolites dates back to the 1960s-1970s and was, from the outset, closely associated with mass spectrometry[201-206]. Studies on the GC determination of catecholamines in the absence of MS detection during the 1970s constitute a separate branch that did not shape the main direction of the field[207,208], a limitation that many authors attributed to the lack of optimal chromatographic columns. As an intermediate line of development, gas-liquid chromatography of these compounds also progressed during the 1970s[209,210]. During the

1980s, the relative merits of gas versus liquid chromatography for catecholamine analysis continued to be debated[211]. However, from the early 1980s through the 2000s, advances in the GC analysis of catecholamines-irrespective of the detector employed-were largely driven by the introduction of capillary columns[212-214].

From the mid-1980s onward, GC-MS methodologies incorporating real-time tracking and monitoring of selected ions, including negative ions, were introduced for catecholamine analysis[215,216]. With the development of thermodesorber technology and microwave-assisted derivatization, sample preparation and conversion of analytes into GC-compatible forms were simplified[217], which also had a favorable impact on GC–MS-based catecholamine assays.

In the 2010s, GC–MS and GC–MS/MS methods for catecholamines and their metabolites became widely adopted in laboratory and clinical practice, typically as routine analytical procedures accessible to mid-level laboratory personnel. At the same time, the accuracy of mass-spectrometric measurements increased with the introduction of new detector technologies, particularly high-resolution Orbitrap ("Makarov trap") analyzers. Among many examples of GC–MS and GC–MS/MS applications to catecholamine analysis are the studies by Zoerner, et al. and Nguyen, et al.[218,219].

8. Historical development of gas chromatography and GC-MS for hydroperoxides

The history of the gas-chromatographic analysis of hydroperoxides, as well as their use in gas chromatography, apparently dates back to the 1970s[220,221]. By the early 1980s, capillary columns were already being actively employed in this area[222] and by the end of that decade the field had joined the broader trend toward integrating gas chromatography with mass spectrometry[223]. Work along these lines continued through the 1980s[224] and into the 1990s[225-227]. During this period, attempts were made both to integrate and to compare GC–MS with GC using flame-ionization detection[228], as well as to integrate and benchmark GC–MS (including ion-trap detection, GC-ITDMS) against high-performance liquid chromatography for these analytes[229]. The 1980s–1990s also saw the evaluation of a number of relatively "exotic" approaches in this analytical domain, including on-column injection[230].

By around 2000, numerous specialized columns and kits optimized for hydroperoxide determination had been introduced and validated, enabling their reliable detection even without mass-spectrometric detection[231-233]. This trend matured into an optimized, quantitative framework by the mid-2010s[234] and was further supported by chemometric tools, offering a low-cost solution for routine, high-throughput laboratories where instrumentation may be limited to GC with an FID detector[235]. However, such approaches are not suitable for operation with a thermal conductivity detector because of the well-known limitations of that detector. In better-equipped settings operating under good laboratory practice (GLP), mass-spectrometric detection is generally considered preferable for hydroperoxide analysis, often in conjunction with cross-validation against other methods such as thin-layer chromatography (TLC)[236,237]. At a minimum, widely available and technically straightforward MS instrumentation employing electron ionization is sufficient for these purposes[238].

9. Historical development of gas chromatography and GC-MS for phosphatidylcholine

There are fewer publications on the gas-chromatographic analysis of phosphatidylcholine than proponents of classical GC might wish. Beginning in the 1970s with gas–liquid chromatography[239], this line of work rapidly shifted its emphasis toward GC-MS, primarily targeting acetolysis products[241]. Thereafter, methodological development progressed only modestly, broadly following the patterns outlined above for the other compound classes considered here. In the 2010s-somewhat later than for the other analytes discussed-GC-MS applications expanded to include "nontraditional" extraction techniques such as headspace solid-phase microextraction (HS-SPME)[242]. A distinctive feature of the GC-related trajectory for phosphatidylcholine has been its integration with and comparison to, matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS)[243].

10. History of the preparation of the present article

The present paper is based on several previously unpublished documents and, in some cases, on the corresponding transcripts of presentations delivered at bibliographic seminars:

- A bibliographic annotated list (used as a working outline) on chromatographic methods for a range of neurotransmitters (covering publications up to the 2010s), compiled and used by the second author during dissertation work, as well as earlier purely abstracting material-largely historical and science-historical in nature-on the development of gas chromatography of neurotransmitters up to the 1990s (prepared during the pre-graduation period while working with outdated instrumentation, under the supervision of a specialist in biogenic monoamines). The contribution of these sources to the present work is estimated at approximately 30%.
- A bibliographic annotated list on gas chromatography and potentiometry in antioxidant chemistry, prepared during an attempt to organize RD on position-sensitive redox-metric measurements and gasometric microscopy at the Institute of Chemical Physics of the Russian Academy of Sciences (2011-2012). This work was discontinued due to termination of the contract and the lack of staff positions in the Department of Dynamics of Chemical and Biological Processes. This source accounts for approximately 10% of the bibliography used here.
- A bibliographic annotated list on applications of gas chromatography in neurochemical research, compiled during the second author's employment in the Laboratory of Neuronal Brain Structure, Department of Brain Research, Scientific Center of Neurology of the Russian Academy of Medical Sciences (2012-2013). Unfortunately, by the mid-2010s these research plans were disrupted: following the reform of the Russian Academy of Sciences, the Laboratory of Neuronal Brain Structure was soon dissolved and the remaining chromatographic equipment (partly purchased by the authors with personal funds) was disposed of. The contribution of this source is up to 20%.
- A bibliographic annotated list on neurochemical applications of GC–MS (maintained in several versions and updated annually from 2013 to 2016 during the second author's work in a mass-spectrometry laboratory at the Institute of Energy

Problems of Chemical Physics of the Russian Academy of Sciences), as well as a similar frequently updated outline for antioxidant-related studies. These lists often included other mass-spectrometric approaches discussed in a comparative context. The contribution of these sources to the present work is estimated at approximately 30%. Unfortunately, owing to the obsolescence of the VARIAN- and Finnigan MAT–based GC–MS instrumentation at the institute, these approaches were not implemented there in routine practice.

- An updated (relative to item 2) bibliographic annotated list on GC and GC–MS in studies of neurotransmitters, bio antioxidants and antibacterial drugs, compiled with active participation of all authors. This list was prepared in the early 2020s for the management of the Department of Dynamics of Chemical and Biological Processes of the Federal Research Center for Chemical Physics of the Russian Academy of Sciences (formerly the Institute of Chemical Physics, RAS) and for the Laboratory of Liquid-Phase Oxidation of the same center. However, due to reorganization processes and redistribution of laboratory space, this document ultimately became unused. The equipment preserved by the authors was subsequently disposed of during the takeover of the premises (room 164) and only partly moved to unsafe rooms in Building 1 of the center, where a ceiling collapse later occurred. In view of the accelerating degradation of the GC/GC–MS infrastructure and the associated laboratory facilities, the authors jointly decided to publish an abridged version of the review material in its current form (without converting the accumulated bibliographic groundwork into empirical GC or GC–MS articles corresponding to the substance-specific sections of the review). We do not anticipate that we will be able to continue work in this direction; therefore, lacking support from a number of colleagues, we are not attempting to upgrade this review note to a full-scale, high-impact review article (in which citing those colleagues would be appropriate). Instead, we present what can reasonably be published given the material and technical resources available (and unavailable) to us.

11. Perspectives and Challenges (Discussions with Students)

A practically important challenge in contemporary redox medicine is not merely to use antioxidants, but to reliably and reproducibly detect and quantify antioxidant compounds and the associated redox markers in real samples: natural matrices (plant raw materials, foods), pharmaceutical substances and precursors, as well as human biomaterials (tissues, blood, urine, saliva, exhaled breath condensate and others). In European and U.S. research agendas, this is directly linked to a shift from "descriptive redox biology" toward measurable redox medicine and precision medicine, where decisions are grounded in validated biomarkers, standardized preanalytical procedures and interlaboratory-comparable data. Without objective assessment of the redox status of biosystems (and its temporal dynamics), it is impossible to develop a constructive approach to what may be broadly termed redox pathology, redox etiology and redox prevention: one cannot properly evaluate the contribution of oxidative stress, compare patient cohorts, rationally select an intervention strategy (diet, nutraceuticals, pharmacological antioxidants) or monitor treatment effects.

Contemporary experience from clinical trials of antioxidants has shown that "universal" regimens often yield equivocal outcomes precisely because interventions are implemented without sufficient characterization of baseline redox profiles and without monitoring of target molecular changes. This underscores the pivotal role of analytical chemistry and analytical biochemistry technologies for antioxidants: they provide the measurement framework for formulating redox-medicine problems, comparable in importance to the role that standardized hormone panels have played in endocrinology and that lipid profiles and high-sensitivity troponin have played in cardiology.

It is therefore important to distinguish between methods that evaluate "overall activity" and those that identify specific molecules. A number of widely used approaches capture integral reducing-oxidizing capacity or a global response (e.g., potentiometric measurements of redox potential, certain assays of total antioxidant capacity). Such methods are useful for screening; however, they are generally nonselective with respect to composition and poorly resolve the contributions of individual redox couples, oxidation products, metal complexes and matrix effects. In a clinical and biological context, this means that what is measured is not the "set of chemical systems actually operating in a given environment," but rather an aggregate signal that may be identical despite fundamentally different molecular causes.

The modern redox concept assumes that many antioxidant mechanisms operate in microheterogeneous and ultramicroheterogeneous environments: membranes, lipoproteins, mitochondria, peroxisomes, the endoplasmic reticulum, granules and microdomains differing in ionic strength, pH, dielectric permittivity and local metal availability. Biological tissue with its organelles and compartments is a particularly illustrative example. Consequently, additive (essentially "summative") assessments and inferences about "overall redox efficacy" are insufficiently rigorous for redox medicine: they do not allow observations to be linked reliably to mechanism and, ultimately, to clinical decision-making.

A minimally appropriate analytical level is the separate quantitative determination of key redox components and markers (e.g., individual low-molecular-weight antioxidants, their oxidized/reduced forms, lipid peroxidation products, oxysterols, markers of carbonyl stress and the like). The most advanced level is position- and compartment-sensitive analysis-i.e., measurement performed precisely where the reaction process unfolds (within an organelle, in a membrane, in the extracellular space or in a specific cell type). This task already belongs to the domains of cytochemistry, spatially resolved biochemistry and ultrastructural approaches and it requires specialized sample preparation and imaging methods; it may therefore fall outside the scope of the present review.

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