Findings in redox biology: From H₂O₂ to oxidative stress

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My interest in biological chemistry proceeded from enzymology in vitro to the study of physiological chemistry in vivo. Investigating biological redox reactions, I identified hydrogen peroxide (H₂O₂) as a normal constituent of aerobic life in eukaryotic cells. This finding led to developments that recognized the essential role of H₂O₂ in metabolic redox control. Further research included studies on GSH, toxicological aspects (the concept of “redox cycling”), biochemical pharmacology (e.g., selenium, carotenoids, flavonoids), and the concept of “oxidative stress.” Today, we recognize that oxidative stress is two-sided. It has its positive side in physiology and health in redox signaling, “oxidative eustress,” whereas at higher intensity, there is damage to biomolecules with potentially deleterious outcome in pathophysiology and disease, “oxidative distress.” Reflecting on these developments, it is gratifying to witness the enormous progress in redox biology brought about by the science community in recent years.

On the sunny side

Born in 1942 in Goslar, the town of Kaiser Barbarossa, I grew up together with my younger brother Eckhart at the Western rim of the Harz mountains in the nearby small city of Seesen in Northern Germany. Ernst Sies, our father, was then working at “Sonnen-Werke” (Sun Company), a food company, of which he was to become CEO after the Second World War. He was fortunate not to be in military action, because maintenance of food production and distribution was essential. When in 1945 the line between the British and the Soviet occupation zones (Iron Curtain) was drawn, it went through the middle of the Harz mountains: we had the good fortune to be on the “sunny side,” just about 25 miles to the west of it…

Living near meadows and hillside forests, I was exposed early to the delights of nature, which made lasting impressions. My parents, Ernst and Ilse Sies, fostered my sense of appreciation for the wonderful treasures provided by nature, as did my first teacher in Elementary School, which I entered in 1948. This teacher, Georg Henkel, contributed a great deal to my positive outlook and confidence: he had a group of us first-graders sit across from each other at a table, gave us problems to solve, such as venturing out to the forest and collecting certain plants and then drawing them in color, doing flower pressings, and presenting the group’s work to the class. It was fun! The class model fostered curiosity and recognized individual contributions. Bottom line: you can find out things for yourself, digest what you find, and get recognition for your achievements even though you will make mistakes. I still have contact with Georg, who is approaching his 99th birthday these days.

The Elementary School in Seesen happened to be where the convoys of US troops made their stop on the way from Frankfurt to Berlin. GIs came over to the school fence and handed us Wrigley’s chewing gum, playing great jazz tunes from the American Forces Network (AFN) radio. The experience shaped my interest in the Anglo-American outlook. A self-assembly ship made of balsa wood from a US care package for Christmas 1948 also made a lasting impression.

Another “sunny side” was the Jacobson-Gymnasium in Seesen, founded in 1801. We had good teachers in Latin, physics, math, geology, literature, and history. The school was equipped with a Steinway grand piano. (Heinrich E. Steinweg, later known as Henry Steinway, was from near Seesen, where he built his very first piano in 1836. His son William donated the Steinway Park in Seesen in 1893. Influenced by this early impression, after receiving a professorship position at Düsseldorf, my first larger purchase was a Steinway; to this day, I enjoy practicing and playing on it at home.)
In 1959, I was one of about 200 students who sailed on the Greek liner *Arkadia* with the Michigan Council of Churches exchange program for a year in the US, selected by the “Youth for Understanding” committee. My first destination was to live with the family of a country doctor and his wife, David P. and Marie Ward, in Pleasant Plain, Ohio, near Cincinnati, where deep conversations contributed to my interest in basic biomedical research. The second half of the exchange year was with Edward and Eleanor McBroom in Kankakee, Illinois, south of Chicago. Son of a state senator, Ed was active in politics, and I am very thankful to the McBrooms for exposing me to another realm of life, again very much “sunny side.” On weekends, we flew in a Cessna to Meigs Field in Chicago on the lakefront of Lake Michigan and listened to Oscar Peterson playing at the London House.

My path in biochemistry and molecular biology

The sequential lines of my research activity—how did they come about, and how did I get into biochemistry (1)? What happened between then and now (Fig. 1), and what were the influences? In the early 1960s, when I entered university as a medical student, redox biochemistry, which would become the research focus throughout my career, was a flourishing field. Reduction-oxidation reactions are at the core of fundamental life processes. The discovery of oxygen was in the 18th century (e.g. Scheele, Priestley, Lavoisier). Energy conversion of sunlight employs redox processes in photosynthesis, and oxidation reactions drive aerobic metabolism. Hydrogen peroxide (Thénard) and selenium (Berzelius) were discovered in the early 19th century, and the elucidation of respiration and redox metabolism occurred in the early 20th century (Warburg, Wieland, Krebs, Szent-Györgyi). Based on major discoveries from the previous decades, many new questions had become answerable. As young ones, we were fascinated by the recent breakthroughs.

After graduating from Jacobsen-Gymnasium in 1961, I started in academia by enrolling at the University of Tübingen as a medical student and at the *studium generale* at the Leibniz-Kolleg, which provided a broader base in science and humanities. (The first curriculum of biochemistry worldwide was introduced at Tübingen in 1962; before that, one would study either chemistry or medicine to embark in biochemistry). The trimester theme was “symmetry,” with excellent young docents from disciplines as far apart as logic, crystallography, and sociology. For example, one of the docents was Ralf Dahrendorf, later director of the London School of Economics. Experimentally, I was asked to determine the optical rotation of a then-uncharacterized rare sugar by polarimetry, a seemingly pedestrian first step into biochemistry. Tübingen University was a bustling place. One of my fellow students was Bert Sakmann, from Stuttgart. We met on the tennis court and went sailing together at Lake Constance. In our discussions on how to continue our studies, we noted the attractiveness, scientific and otherwise, of Munich, and we enrolled at the Ludwig-Maximilians-Universität. Bert leaned to physiology, joining the Creutzfeld neurophysiology laboratory at the Max Planck Institute of Psychiatry. His studies led him to develop an exciting pathway for fundamental research, the patch-clamp technique; he was awarded a Nobel Prize in Physiology or Medicine in 1991. I chose to go into biochemistry. Theodor Bücher (Fig. 2), a student of Otto Warburg’s, was about to take over the chair that had been held by Adolf Butenandt. Bücher was still at the University of Marburg, where he had laid fundamental ground in clinical enzymology and had helped found the scientific basis of the Eppendorf Co. at Hamburg as well as the Boehringer Biochemicals Co. at Tutzing. His group developed ingenious methods in analysis, including what is known as the “Eppendorf cup” and the microliter pipette system with the disposable tip, now worldwide standard laboratory equipment. I approached Professor Bücher to ask whether he would accept me as a doctoral
I would like to offer a few words on the stimulating scientific atmosphere at Munich. I had moved to the Max-Kade-House, a student dormitory, which had been donated by the German-American philanthropist Max Kade. Werner Heisenberg had his home across the street, bordering the park, Englischer Garten. He had his daily walk through the park to his nearby Max Planck Institute of Physics and Astrophysics, and I first chatted with him on a walk along the park. In 1964, Heisenberg was the patron of a meeting of the “Deutsche Gesellschaft für Naturforscher und Ärzte” (German Society of Scientists and Physicians) at Weimar, at the other side of the Iron Curtain, then East Germany; I was among the few students from the West to be able to participate. At Munich, there was no formal study of biochemistry yet, so parallel to my experimental thesis work, I enrolled in an autodidactic fashion in the organic-chemical laboratory course and in the biochemical colloquium by Feodor Lynen (Nobel laureate for his work on fatty acid metabolism) and courses of electronics and biomathematics. Also, there were great seminars on behavioral physiology by Konrad Lorenz at nearby Seewiesen and on circadian rhythm by Jürgen Aschoff at Erling-Andechs. This brief description of the scientific atmosphere at the time may illustrate how it boosted interest and fostered curiosity.

In 1963, Bert and I had an exciting time, participating as students in the 13th Nobel Prize Winners Meeting at Lindau, on Lake Constance. It was fascinating to meet and talk to eminent scientists, after a grandiose opening by Count Lennart Bernadotte on the Isle of Mainau. Great biochemists were in attendance: Hugo Theorell from Stockholm, talking on ethanol combustion in the liver; Severo Ochoa from New York, on the chemical basis of heredity; Otto Warburg from Berlin, who was in a way my “scientific grandfather-to-be,” on the chemistry of photosynthesis; Sir MacFarlane Burnet from Melbourne on the role of thymus in immunity; and last but certainly not least, Sir Hans Krebs from Oxford, who in later years would become a close personal friend and mentor (3), on the regulation of cell metabolism. Wonderful physicists and chemists were also present and available for a brief chat, notably Max Born from Bad Pyrmont and Otto Hahn from Göttingen. It is, of course, difficult to assess what the direct personal exposure to such “Olympic” figures in science does to young students and their own future outlook, but I strongly believe it is a most positive event. (The Lindau Meetings of Nobel Prize Winners are still thriving, headed by Countess Bettina Bernadotte. Later I had the good fortune to be a member of the Council and its Vice President from 2005 to 2011, helping to select the young scientists from around the globe and shaping the rich scientific program for interactions with the Laureates at Lindau.)

With an interim clinical semester in 1964–65 at the Sorbonne at Paris (Hôpital Cochin, Salpetrière), I finished clinical studies at Munich and spent residency time in clinical medicine at the university hospital in Tübingen (Prof. H. E. Bock) and at small provincial hospitals. Bücher offered me a postdoc position to return to the Munich institute; he gave me liberty to choose my research topic. We organized a Mosbach Colloquium on “Inhibitors—Tools in Cell Research” in 1969 (4), and we had invited Otto Warburg to attend. Warburg regretfully...
declined, saying: “I have already attended a meeting this semester!”

Otto Wieland and Benno Hess organized informal meetings at Hochhausen Castle on the Neckar river, inviting a few young biochemists for lectures with no slides, just chalk and blackboard, with intense discussion. The castle did not have enough rooms, and I recall sharing a double-room with Detlev Riesner (he later founded the bioanalytics company Qiagen after his move to Düsseldorf).

Several groups in Munich became interested in oxygen-related topics, ranging from medicine to biochemistry, toxicology, nutrition research, botany, and radiation chemistry. We decided to meet for cross-discipline discussions and in 1977 founded the “Münchner Sauerstoffclub” (Munich Oxygen Club) at an appropriate place: the Max-Emanuel Brewery (briefly described in Ref. 5). This was perhaps one of the first of many “oxygen clubs” around the world.

**Hydrogen peroxide (H_{2}O_{2}) as a normal constituent of aerobic metabolism**

How did this topic become of interest? Focusing here on my background mindset, the answer is 2-fold: a prepared mind and curiosity, plus some serendipity.

**Prepared mind**

Bücher and Klingenberg had published a masterpiece article in *Angewandte Chemie* on the organization of living cells in 1958 (6). Their work remains central to cell physiology still today. Otto Warburg had noted already in 1928 that one should “…study enzymes under the most natural conditions of action, in the living cell itself. From the standpoint of preparative chemistry they may be looked upon as being of the utmost impurity. However, if one finds reactants that selectively react with the enzymes, the rest of the cell interferes as little as does the glass wall of a test tube in which a chemical reaction is carried out” (7). Bücher’s group had developed the experimental system of the isolated perfused rat liver, maintaining its normal metabolism at physiological capacity for hours. Importantly, organ spectrophotometry made it possible to monitor a non-invasive readout of ongoing metabolic processes in the intact organ. Bolko Brauser, a congenial biophysicist and senior assistant in the laboratory, had adapted a rapid-scanning spectrophotometer, the “Rapidспектroskop,” for supersensitive differential spectrophotometry (8, 9). I joined Brauser, and we investigated the redox state of mitochondrial cytochromes and cytochrome P450, the cellular heme proteins with a prominent absorbance band in the blue spectral region, the Soret band (10, 11).

**Curiosity**

Given the opportunity to examine cellular physiology noninvasively with very high sensitivity at all the wavelengths from blue to red and beyond, I wondered whether other heme proteins could be analyzed. Catalase and heme peroxidases came to mind. How to “pin them down,” how to follow their action? Mitochondrial cytochromes and cytochrome P450 were detectable by their redox transitions (e.g. becoming reduced when oxygen became limiting in hypoxia or anoxia). Would it be possible to detect catalase Compound I as distinct from catalase, as one could distinguish reduced from oxidized cytochrome P450? This would provide proof that H_{2}O_{2} exists in the normal cell!

**Problem**

For a long time, identification and characterization of H_{2}O_{2} in eukaryotes had been challenging; early attempts by Heinrich Wieland to detect H_{2}O_{2} in animal metabolism had failed (12). Chance had noted that “quantitative evidence for the existence of significant amounts of … H_{2}O_{2} in tissue is lacking, since catalase, by virtue of its peculiar capacity for catalatic reactions… literally ‘destroys the evidence’ of free hydrogen peroxide in the cell” (13). Attempts by several groups to identify H_{2}O_{2} in intact cells by monitoring the Soret band of catalase Compound I had remained futile, largely because of scattering artifacts and low signal-to-noise ratios.

**Solution**

The breakthrough came by employing noninvasive organ spectrophotometry in the near-IR region, the spectral area where light scattering is far lower than at the Soret band spectral region. Catalase Compound I has a charge-transfer band with an absorbance peak at 660 nm in the difference spectrum with catalase (14). So I remember one late evening in 1969 in the basement laboratory in Munich at the Rapidспектroskop when I decided to take a look at the near-IR, using the dual-wavelength difference between 660 and 640 nm to cancel out noise and then inject ethanol, a known hydrogen donor for the peroxidatic reaction of catalase, at low concentration to the hemoglobin-free perfused rat liver. Hooray, it worked! There was a swift deflection, and after I stopped the ethanol infusion, the signal returned to the original level. The signal also responded in normoxia-anoxia transitions, and the two transitions were not additive (Fig. 3). A steady-state level of catalase Compound I was identified. This proved the existence of normal H_{2}O_{2} production in the intact eukaryotic cell, a slightly heretical thought at the time. After discussing it with Britton Chance on one of his visits to Munich and completing appropriate control experiments (e.g. the complete absence of the deflections shown in Fig. 3 when the animals had been pretreated with the catalase inhibitor 3-amino-1,2,4-triazole), I published jointly with Chance in the then-new *FEBS Letters* (15).

**Sequels**

This opened up a new direction of redox research at the Johnson Research Foundation at Philadelphia (16, 17). Working with Nozomu Oshino, I used steady-state titrations with methanol as hydrogen donor to quantify H_{2}O_{2} generation of about 50 nmol/(min × g of liver), which corresponds to about 2.5% of oxygen uptake (18, 19). The overall concentration of H_{2}O_{2} in the liver cell was calculated to be 10 nM (20) (see Fig. 4), with an estimated physiological range being 1–100 nM (21). Dean Jones further advanced analysis of H_{2}O_{2} metabolism in isolated hepatocytes by spectroscopy of catalase Compound I.
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(22). The timeline of $\text{H}_2\text{O}_2$ in chemistry and biology (Fig. 5) illustrates the development, with further milestones of the peroxiredoxins and the peroxiporins in the 20th century. A new era in $\text{H}_2\text{O}_2$ research began in the 21st century with the introduction, by Vsevolod Belousov, of the OxyR-based genetically encoded fluorescent probe, Hyper, permitting noninvasive readout of $\text{H}_2\text{O}_2$ in subcellular compartments (23, 24).

In 1971, I submitted my “Habilitation” thesis to become recognized as “Privatdozent,” an independent member of the University. Entitled The peroxisome in the hepatocyte: catalase Compound I in hemoglobin-free perfused rat liver, it was published in Angewandte Chemie in 1974 (25).

Nicotinamide adenine dinucleotides, NADPH and NADH

In 1935–1936, Warburg had discovered the nicotinamide adenine dinucleotides (then called pyridine nucleotides, DPNH and TPNH) as coenzymes of dehydrogenases: NADP as coenzyme of glucose-6-phosphate dehydrogenase and NAD$^+$ as coenzyme of fermentation. The pathways of substrate hydro­gen and the differences in redox state between the two systems had been described (6). Using organ spectrophotometry and fluorometry, we were able to follow the redox state of extrami­tochondrial NADPH during cytochrome P450-dependent drug metabolism (11). These were the early days in the drug metabolism field, and at a workshop in Konstanz in 1968, I met Lars Ernster and Sten Orrenius from Stockholm, forming the basis for a lifelong friendship and many scientific interactions. We found that mitochondrial NADPH was oxidized during ammonia metabolism (26, 27). Work with Dieter Häussinger, one of my early doctoral students, led to the identification of flux regulation of glutamine synthase and glutaminase and to the description of cell heterogeneity, or zonation, in the liver (28). These observations stimulated research in experimental hepatology; Dieter became a prominent clinician and hepatologist, and he also founded an Institute of Tropical Medicine in Ethiopia. Metabolic compartmentation became amenable for research, and we continued to investigate nicotinamide nucleotide compartmentation (summarized in Ref. 29).

GSH, selenium

Turning back to $\text{H}_2\text{O}_2$, catalase was not the only enzyme reacting with $\text{H}_2\text{O}_2$. GSH peroxidase had been discovered (30), and Leopold Flohé at Tübingen had started working on its enzy­mology. In collaborative work, we showed in perfused liver that hydroperoxides indeed led to oxidation of GSH to the di­sulfide GSSG, that the reaction utilized NADPH, and that GSSG was released from the liver (31). Albrecht Wendel, a doc­toral student of Flohé’s, joined our endeavor, and we had great times in our GSH research. Two international conferences on GSH testify to this, one at an exquisite villa at Tübingen (32) and the other at Reiensburg Castle near Ulm, with Alton Meier and Sir Hans Krebs attending, as well as all of us junior scientists who were starting in research (Ref. 6) (33). Gianna Bartoli, a postdoc from Rome, found that GSH release from the liver exceeded GSSG release by about 10-fold, opening the field of interorgan relationships in GSH metabolism (34). GSSG was released into bile, as were the GSH thioethers, the products of GSH S-transferases, also called S-conjugates (35, 36). In perfused rat heart, Toshihisa Ishikawa, a postdoc from Sapporo, later showed cardiac energy-dependent GSSG and S-conjugate export (37).

Düsseldorf

As it went in German academia, a time came to wander off. In contrast to today’s tenure track career pathway, one needed to be recruited from another university to become Full Professor and Chairman, rather than “moving up the ladder” internally. In 1978, the chair at the Institute for Physiological Chemistry at the University of Düsseldorf was announced, and I applied. My first visit to that campus had been during the 1974 meeting of the German Society for Biological Chemistry, to speak in a session chaired by Sir Hans Krebs, related to gluta­mine metabolism. The talk had ended and, in his low voice, Sir Hans said that he recalled having done a similar experiment in 1935! This taught me a lesson to go back to read the literature more deeply. (Nowadays, we may miss important literature if we depend solely on a superficial search by an easy click on PubMed).

Redox cycling

A variety of compounds, such as quinones, iron chelates, and aromatic nitro compounds, can undergo one-electron reduc­tion at the expense of NADPH, followed by autoxidation. To­gether with Hermann Kappus, we introduced the concept of “redox cycling” (38). Several anticancer agents, and also some mutagens, operate on this principle. We examined the protec­tion by NADPH:quinone oxidoreductase, which reduces the quinone by two-electron reduction (39, 40). The resulting hydroquinone then is available for glucuronidation, thus circum­venting redox cycling. The absence of an active NADPH:quinone oxidoreductase (null-allele) is associated with increased cancer incidence (e.g. in urological malignancies) (41).
Ebselen

Having worked on the selenoenzyme, GSH peroxidase, a fortunate contact brought us into the field of organoselenium compounds. Erich Graf, the head of research and development at a pharmaceutical company, Nattermann & Cie., at nearby Cologne, asked us to examine an organic selenium compound first synthesized in 1928, 2-phenyl-1,2-benzoisoselenazol-3(2H)-one, which had shown anti-inflammatory activity in their assays. In the \textit{in vitro} assay for GSH peroxidase activity, we found that the selenium compound exhibited enzyme-like activity, whereas the sulfur analog was inactive (42). The compound received the name ebselen, and we published several papers together. As collaborators from academia and industry, we were awarded the Galenus Prize in 1990 (see Ref. 43). Since this time, the organoselenium field has flourished, with ebselen envisaged as a protein thiol modifier (44, 45). Recently, ebselen was found to most efficiently inhibit the main protease of the coronavirus, SARS-CoV-2, in a screen of 10,000 compounds (46), making it a potential lead compound for treatment of COVID-19 (47). It is also being studied in clinical trials as a lithium mimetic in bipolar disorder and as a lead drug for hearing loss (see Ref. 47). It is gratifying to see renewed interest in ebselen in the clinical setting after our basic research from decades ago.

Singlet molecular oxygen

Enrique Cadenas, originally from Buenos Aires, Argentina, joined our group in 1981 as an Alexander-von-Humboldt Fellow, then coming from the Johnson Research Foundation at Philadelphia. He used a photon counter to set up measurement of low-level chemiluminescence emitted by electronically excited reactive oxygen species. This enabled the detection of the formation of singlet molecular oxygen by its dimol emission in the enzymatic reduction of prostaglandin G2 to H2 (48) and during peroxidation reactions in intact cells (49). Together with Paolo di Mascio, a doctoral student from Brussels, we used a thermolabile endoperoxide to generate singlet oxygen and analyzed its reactions with biological targets (e.g. the formation of single-strand breaks in plasmid and bacteriophage DNA) (50). Using a germanium diode to monitor the monomol emission of singlet oxygen at 1,270 nm, we examined the quenching of singlet oxygen by carotenoids, tocopherols, and thiols (51), finding that lycopene, the red carotenoid in tomato, is the most efficient singlet oxygen quencher (Fig. 7A) (52). Lars-Oliver Klotz,
a postdoc from Tübingen, investigated the role of singlet oxygen in cell signaling, with an emphasis on mitogen-activated protein kinases (p38, JNK, and ERK) (56, 57). Research on singlet oxygen and on excited carbonyls was continued in joint work with colleagues Paolo Di Mascio, Marisa Medeiros, and Etelvino Bechara in Sao Paulo and Jean Cadet in Grenoble (58).

In work with Karin Scharffetter-Kochanek and Peter Brenneisen, we found that singlet oxygen is an early intermediate in induction of interstitial collagenase by UV radiation in skin fibroblasts (59, 60), and with Jean Krutmann we found that it mediates the UV-A–induced generation of the photoaging-associated mitochondrial common deletion (61).

Receptor-mediated superoxide production

A major source of H2O2 comes from the dismutation of the superoxide anion radical (62). As “professional” immune cells, activated leukocytes release superoxide (63). With Beate Meier we found that normal fibroblasts also release superoxide under the control of cytokines, an early observation in the field of redox signaling (Fig. 8) (64).

Nutritional biochemistry: Carotenoids, flavonoids, selenium

Due to their polyene structure, carotenoids quench singlet molecular oxygen particularly well. Wilhelm Stahl joined the laboratory in 1990 as a senior postdoc with experience in industry and started in-depth analysis of nutritional biochemical properties of carotenoids. We established lycopene as a biologically important carotenoid in humans (65), which was later corroborated epidemiologically for certain types of cancer. (As an aside, my contribution to the then emerging field of epidemiology was the nearly perfect association \( r = 0.982 \) of the number of newborn babies with the number of brooding storks, showing what every child in Germany knows: storks bring babies (Fig. 9) (66)! This illustrates that associations can generate interest but cannot prove cause-effect relationships.) Dietary tomato paste protected against UV light–induced erythema in humans (67, 68). We found that lycopene oxidation products stimulated gap-junctional intercellular communication via connexins (69). Together with Alex Sevanian from the University of Southern California, we described the nutritionally induced oxidative responses, “postprandial oxidative stress” (70). Cristina Polidori, a postdoc from Perugia, analyzed the profile of antioxidants in plasma, with emphasis on the age-related diseases (71, 72). Fig. 10 shows members of the group at a laboratory workshop in 2002.

Gavin Arteel, a postdoc from Ron Thurman’s group at the University of North Carolina (Chapel Hill, NC, USA), together with Karlis Briviba and Claus Jacob, worked on protection against peroxynitrite by selenium and tellurium compounds (73, 74). Flavanols isolated from cocoa beans were also found to be effective (75). This led to a longstanding interaction with the cardiology department: Christian Heiss, a joint doctoral student with Malte Kelm from cardiology, found that cocoa flavonols had clear-cut positive vascular effects in human volunteers, demonstrated by an increase in flow-mediated dilation of the brachial artery (as a marker of vascular health) and of circulating plasma protein-nitroso compounds (Fig. 7B) (53). Later, joint work with Hagen Schroeter attributed this effect to \((-\)epicatechin isolated from the cocoa bean (76). High-flavanol cocoa improved the surface profile of the skin (Fig. 7C) (54). With Tankred Schewe we investigated the mechanisms of vascular flavanol effects in view of their anti-
inflammatory action, lowering F₂-isoprostanes and inhibiting 15-lipoxygenases (77–80).

Selenoprotein P, when released by the liver, circulates in the bloodstream and is taken up via receptors by peripheral organs to provide its selenocysteine residues for subsequent synthesis of other selenoproteins. We found that selenoprotein P also covers glycoproteins at the surface of cells by analyzing heparin binding in surface plasmon resonance experiments (81). Together with Holger Steinbrenner, we investigated the mechanisms by which selenoproteins protect against reactive oxygen species, working toward understanding success and failure in the use of selenium for cancer prevention (82, 83). We also described the role of dietary selenium as an adjuvant therapy for viral and bacterial infections, a topic of current interest during the SARS-CoV-2 pandemic (84).

**The concept of oxidative stress: Eustress and distress**

In the early 1980s, research had rapidly progressed from the already comprehensive knowledge in redox reactions that had been presented in our extensive review (20). In an attempt to conceptualize the field, I defined “oxidative stress” as “a disturbance in the prooxidant-antioxidant balance in favor of the former” (85, 86). The underlying concept is that living organisms operate at steady state in an open metabolic system, maintaining a balance of in-flow and out-flow, or homeostasis. The origin of the basic principle of stress and stress responses dated back to Selye in 1936 (87), and the first sentence in Ref. 85 was to state that, “as a biochemist, one may wonder whether Selye’s term should be stressed as it is in the present context.” The experimental evidence supported the thesis that the favorable aspects of aerobic life are also linked to the potentially

![Graph A: Singlet oxygen quenching by carotenoids](image1.png)

**Figure 7. Translation of basic science to human health.** A, singlet oxygen quenching by carotenoids (52). B, improvement of flow-mediated dilation of brachial artery and increase of plasma protein-nitroso compounds by flavanols from cocoa (53). C, skin surface profile improvement after a high-flavanol cocoa drink (54). Compiled in Ref. 55.
dangerous oxygen-linked processes as diverse as inflammation, aging, carcinogenesis, drug action and toxicity, defense against invading organisms, and more. Strategies of antioxidant defense were becoming better-known (88, 89). Also, adaptive stress responses were discovered, notably the newly discovered OxyR regulon in bacteria (90), followed by the eukaryotic NF-κB, HIF, and Nrf2/Keap1 response systems (Fig. 11). Advances in understanding redox regulation, redox sensing, and redox signaling led us to update the concept: oxidative stress is defined as "an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage" (91–93).

It follows from this definition that oxidative stress can range from mild physiological maintenance challenge, called oxidative eustress, to toxic oxidative burden which damages biomolecules, oxidative distress (Fig. 12) (21, 91). Oxidative equivalents utilized in redox signaling target transcription factors directly or indirectly, and H₂O₂ emerged as the central redox signaling molecule in oxidative eustress (21). Aware that H₂O₂ is generated upon low-dose ionizing radiation, we hypothesized that it may mediate hormetic effects of low-dose ionizing radiation (95). The disturbed redox homeostasis in oxidative distress might also be the molecular link between chronic psychological work stress and coronary heart disease (96). H₂O₂ signaling is part of the “redox code,” a set of principles describing the organization of redox biology (97). Having followed research on H₂O₂ since its detection decades ago, it is satisfying to note that, as the major biologically active reactive oxygen species, H₂O₂ is recognized as a versatile pleiotropic physiological signaling agent, fulfilling essential functions in metabolism (94, 98). These are considered to form a basis for a future “redox medicine.” It was a pleasure to convene experts to present current knowledge in this field in a book, entitled Oxidative Stress: Eustress and Distress (99). From enzymology in vitro to physiological chemistry in vivo (100), redox biology has “come a little ways,” exposing a bright future, given the marvelous novel tools of analysis which recently have become available.

**Interactions with redox biologists around the globe**

Karl Popper called science an "unended quest" (101), which very well characterizes the research field of redox biology. Eminent scientists have discussed the creative process in science and medicine (102). I was very fortunate to have witnessed, and be part of, the development of this global activity. Visiting scientists came from all continents to join my group, first in Munich, then in Düsseldorf. Here, I would like to express my appreciation and thanks to the Alexander-von-Humboldt Foundation for longstanding support of numerous
international Awardes and Fellows who visited my laboratory over the years for joint research, among them Chris Foote from UCLA; Dan Ziegler, Austin; Brian Ketterer, London; Gustav Born, London; Keith Ingold, Toronto; Fred Sundquist, UCSD; Govind Mugesh, Bangalore; Greg Bartosz, Lodz. (In later years, I served on the Alexander-von-Humboldt review and selection panels for over a decade.) I am also thankful to the Deutsche Forschungsgemeinschaft (DFG), Bonn, and the National Foundation for Cancer Research (NFCR), Bethesda, MD, USA (see below) for longstanding support.

Figure 10. Members of the Düsseldorf group at a laboratory workshop (Schloss Mickeln, 2002).

Figure 11. Timeline showing the concepts of stress and stress responses. Mithridates VI and Paracelcus had early insights. Bernard’s concept of milieu interieur received the name “homeostasis,” and the Arndt–Schulz rule received the name “hormesis.” The 20th century brought the adaptive stress response, heat shock response, oxidative stress, OxyR, allostasis, unfolded protein response, NF-κB, HIF, and Nrf2/Keap1. From Ref. 91, where the literature on these concepts is listed.
A wonderful description of my worldwide scientific contacts and friendship is given in a volume with over 30 contributions, initiated by the publisher, Anthony Newman, and assembled by the Editors Henry Forman and Shinya Toyokuni (103). The interactions with my Japanese colleagues have been written up (104), and many similar close relationships still need to be formally acknowledged, with fruitful research visits by colleagues from the US (Jim Kehrer, UT Austin; Frank Meyskens, UC Irvine; Jim Thomas, Iowa State University), and interactions with colleagues from Spain (José Vina, Francisco Romero, José Estrela, Santiago Lamas), Italy (Mario Comporti, Angelo Benedetti, Alfonso Pompella, Giuseppe Poli, Fulvio Ursini), France (Jean Cadet, Alain Favier, Jean-Marie Aubry, Josiane Cillard, Ingrid Emerit), Switzerland (Wim Koppenol, Angelo Azzi), and South America (Giuseppe Cilento, Etelvino Bechara, Federico Leighton, Lionel Gil, Rafael Radi), to name a few.

Most of these relationships stemmed from personal interactions at scientific meetings.

The colleagues organizing such events deserve immense credit. I just want to mention one line of development, which influenced my own path in science considerably. In the early 1970s, the heyday of “free radicals” (105), Franklin Salisbury of Bethesda, Maryland, founded the National Foundation for Cancer Research (NFCR) to provide funds for Albert Szent-György’s laboratory at Woods Hole, Massachusetts. Trevor Slater from Brunel University in the UK had developed an interest in lipid peroxidation and had established contacts to research groups in Italy and Austria. He had also agreed to serve as a project director for the NFCR. Slater, together with Robin Wilson, inaugurated the Society for Free Radical Research (SFRR) in the UK in 1982. (In later years, I participated in shaping this worldwide society, serving as President of SFRR-International from 1998 to 2000.) In the US, Lester Packer at the University of California (Berkeley, CA, USA) inaugurated the Gordon Research Conference on Oxygen Radicals in 1981. In 1983, the second Gordon Research Conference on this topic took place in Ventura, California. Thereafter, a small NFCR workshop was held at Montecito, California, with Szent-György, Salisbury, and a handful of colleagues, convened by Trevor Slater and Lester Packer (Fig. 13). Fortunately, I was one of them (again, “sunny side”), which was consequential in two respects: (i) it established my relationship with NFCR (motto: “Laboratory without Walls”), resulting in research funding in 1984, which lasted throughout the years until 2016, for which I am most thankful; and (ii) it led to a sabbatical at Berkeley in 1984/1985 with Bruce Ames, Lester Packer, and Martyn Smith (Fig. 14). This was truly “sunny side,” as I also met my future wife, Nancy, who then was completing her Ph.D. work at the Berkeley neuroendocrinology laboratory of Paola Timiras.

Lester Packer, with his gregarious personality and extraordinary enthusiasm, attracted colleagues from around the world for workshops in the redox field, an activity that grew into the founding of the “Oxygen Club of California” (OCC), which held biennial meetings in California (Fig. 15), Oregon (with Balz Frei
at the Linus Pauling Institute in Corvallis), Spain, and Italy and sponsored meetings at many other places on all continents, contributing to the development of redox biology worldwide. With Enrique Cadenas, John Maguire, Maret Traber, and Chandan Sen, I gladly served on the Board of Directors of OCC, shaping various OCC meetings together with Cesar Fraga, Patricia Oteiza, Giuseppe Poli, Juan Sastre, and Giuseppe Valacchi.

In 1998, the Institute of Medicine of the National Academies held a meeting of the Panel of Dietary Antioxidants (Chair, Norman Krinsky) in Washington, D.C., at which I gave a survey lecture. In the audience there was Harold Schmitz, whom I had known from his dissertation work on carotenoids with John Erdman at Champaign-Urbana. Working by this time at the Hackettstown, New Jersey, facility of Mars, Inc., Schmitz had recently embarked on identifying procyanidins and flavonoids in cocoa. This was the starting point of our long-term joint research in nutritional biochemistry, which led to the clinical studies in cardiovascular medicine, referred to above (Fig. 7B).

In acquiring seniority in science, one takes on responsibilities to serve the next generation (106). I did so in several ways. I took active part in the Academy of Sciences and Arts in our state, Northrhine-Westphalia, serving as its President from 2002 to 2005. One project was to introduce the “Junges Kolleg” (Young College), where young scientists with outstanding achievements are elected to join as junior members of the academy and are provided with funds and no-strings-attached freedom of research activities. By now, many of them have become successful professors in their respective fields.

After stepping down as professor and chairman at the Institute of Biochemistry and Molecular Biology at Heinrich-Heine-University Düsseldorf in 2008, as an emeritus professor I had the good fortune to be informally appointed as research professor by our rector and as senior scientist at the Leibniz Research Institute for Environmental Medicine, positions that I hold to this day. Also, I served for a few years as visiting professor of biology and biochemistry at the College of Science of King Saud University in Riyadh, Saudi Arabia. As a council member of the Starck Foundation in Germany, I have helped support Jewish students with university scholarships. A really unique and wonderful characteristic of science is the continuing progress into the previously unexplored—it never stops (“unended quest,” mentioned above), and so I am very content observing the advances in redox biology from my current vantage point (Fig. 1).

Appreciation

Happenstance and serendipity play a role, but what one needs to be particularly thankful for is the initial trust and guidance by family, teachers, mentors, and colleagues, starting in early childhood all the way through to university, academia, and beyond. I was lucky to have had caring parents and an
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Figure 14. Sabbatical at University of California (Berkeley, CA, USA), 1984/1985. *Left to right:* Lester Packer, Bruce Ames, Helmut Sies, Martyn Smith.

Figure 15. Oxygen Club of California (OCC) Conference (Santa Barbara, CA, USA), 2002. Shown are Enrique Cadenas (*standing*) and Adjunct Professors (*left to right*) Lester Packer, Alberto Boveris, Helmut Sies, and Catherine Rice-Evans (University of Southern California).
intact home, stimulating foster parents overseas, and, of pivotal importance, academic mentors. I can only hope that during my own career as a university professor and research scientist, I was able to generate the feeling of enthusiasm and support in my students and associates to help their creativity and development unfold in science. I thank Andreas Reichert, my successor, for his collegiality and friendship. I would like to pay special tribute to a close personal friend and mentor who had much to do with my own path in the world of science: Gustav V. R. Born. I was fortunate to know this superbly generous scientist who helped shape scientific careers in all parts of the world, including many in Germany. I also would like to acknowledge my students and associates to help their creativity and development unfold in science. Special thanks go to my wife, Nancy, for her warmth and continuous understanding, help, and support.

Conflict of interest—The author declares that he has no conflicts of interest with the contents of this article.

References

1. Sies, H. (2007) How I became a biochemist. IUBMB Life 59, 469–473 CrossRefMedline
2. Bücher, T., and Sies, H. (1969) Steady state relaxation of enolase in vitro and metabolic throughput in vivo of red and white rabbit muscles. Eur. J. Biochem. 8, 273–283 CrossRefMedline
3. Krebs, H. A. (1981) Reminiscences and Reflections. pp. 1–298, Clarendon Press, Oxford
4. Bücher, T., and Sies, H. (eds) (1969) Inhibitors—Tools in Cell Research, pp. 1–415, Springer, Heidelberg
5. Del Río, L. A. (2015) ROS and RNS in plant physiology: an overview. J. Exp. Bot. 66, 2827–2837 CrossRefMedline
6. Bücher, T., and Klingenberg, M. (1958) Wege des Wasserstoffs in der lebenden Organisation [Pathways of hydrogen in the living organization]. Angew. Chem. 70, 552–570 CrossRef
7. Warburg, O. (1928) Über die katalytischen Wirkungen der lebendigen Substanz [On the catalytic actions of the living substance]. Springer-Verlag, Berlin
8. Brauser, B. (1968) Ein Gerät zur höchstempfindlichen Differentialspektrophotometrie mit dem Rapidsspektrotop [An apparatus for highest-sensitive differential spectrophotometry with the Rapidsspektrotop]. Z. Analyt. Chem. 237, 8–17 CrossRef
9. Sies, H., and Brauser, B. (1980) Analysis of cellular electron transport systems in liver and other organs by absorbance and fluorescence techniques. Methods Biochem. Anal. 26, 285–325 CrossRefMedline
10. Sies, H., Brauser, B., and Bücher, T. (1969) On the state of mitochondria in perfused liver: action of sodium azide on respiratory carriers and respiration. FEBS Lett. 5, 319–323 CrossRefMedline
11. Sies, H., and Brauser, B. (1970) Interaction of mixed function oxidase with its substrates and associated redox transitions of cytochrome P-450 and pyridine nucleotides in perfused rat liver. Eur. J. Biochem. 15, 531–540 CrossRefMedline
12. Wieland, H. (1925) Über den Mechanismus der Oxidationsvorgänge. IX. [On the mechanism of oxidation processes. IX]. Justus Liebig’s Ann. Chem. 445, 181–201 CrossRef
13. Chance, B. (1951) Enzyme-substrate compounds. Adv. Enzymol. Rel. Subj. Biochem. 12, 153–190 CrossRefMedline
14. Brill, A. S., and Williams, R. J. P. (1961) Primary compounds of catalase and peroxidase. Biochem. J. 78, 253–262 CrossRefMedline
15. Sies, H., and Chance, B. (1970) The steady state level of catalase compound I in isolated hemoglobin-free perfused rat liver. FEBS Lett. 11, 172–176 CrossRefMedline
16. Chance, B., and Oshino, N. (1971) Kinetics and mechanisms of catalase in peroxisomes of the mitochondrial fraction. Biochem. J. 122, 225–233 CrossRefMedline
17. Boveris, A., Oshino, N., and Chance, B. (1972) The cellular production of hydrogen peroxide. Biochem. J. 128, 617–630 CrossRefMedline
18. Oshino, N., Chance, B., Sies, H., and Bücher, T. (1973) The role of H2O2 generation in perfused rat liver and the reaction of catalase compound I and hydrogen donors. Arch. Biochem. Biophys. 154, 117–131 CrossRefMedline
19. Sies, H., Bücher, T., Oshino, N., and Chance, B. (1973) Home occupancy of catalase in hemoglobin-free perfused rat liver and of isolated rat liver catalase. Arch. Biochem. Biophys. 154, 106–116 CrossRefMedline
20. Chance, B., Sies, H., and Boveris, A. (1979) Hydroperoxide metabolism in mammalian organs. Physiol. Rev. 59, 527–605 CrossRefMedline
21. Sies, H. (1977) Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: oxidative eustress. Redox Biol. 11, 613–619 CrossRefMedline
22. Jones, D. P., Thor, H., Andersson, B., and Orrenius, S. (1978) Detoxification reactions in isolated hepatocytes: role of glutathione peroxidase, catalase, and formaldehyde dehydrogenase in reactions relating to N-demethylation by the cytochrome P-450 system. J. Biol. Chem. 253, 6031–6037 CrossRef
23. Belousov, V. V., Fradkov, A. F., Lukyanov, K. A., Staroverov, D. B., Shakhbazov, K. S., Terskikh, A. V., and Lukyanov, S. (2006) Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. Nat. Methods 3, 281–286 CrossRefMedline
24. Pak, V. V., Ezeriqa, D., Lyublinskaya, O. G., Pedre, B., Tyurin-Kuzmin, P. A., Mishina, N. M., Thauvin, M., Young, D., Wahni, K., Martinez Gache, S. A., Demidovich, A. D., Ermarkova, Y. G., Maslova, Y. D., Shoikhina, A. G., Erolgu, E., et al. (2020) Ultrahighly sensitive genetically encoded indicator for hydrogen peroxide identifies roles for the oxidant in cell migration and mitochondrial function. Cell Metab. 31, 642–653 CrossRefMedline
25. Sies, H. (1974) Biochemistry of the peroxisome in the liver cell. Angew. Chem. Int. Ed. Engl. 13, 706–718 CrossRefMedline
26. Sies, H., Häussinger, D., and Grosskopf, M. (1974) Mitochondrial nicotinamide nucleotide systems: ammonium chloride responses and associated metabolic transitions in hemoglobin-free perfused rat liver. Hoppe Seylers Z. Physiol. Chem. 355, 305–320 CrossRefMedline
27. Sies, H., Summer, K. H., and Bücher, T. (1975) A process requiring mitochondrial NADPH: urea formation from ammonia. FEBS Lett. 54, 274–278 CrossRefMedline
28. Häussinger, D., Sies, H., and Gerok, W. (1985) Functional hepatocyte heterogeneity in ammonia metabolism: the intercellular glutamine cycle. J. Hepatol. 1, 3–14 CrossRefMedline
29. Sies, H. (1982) Nicotinamide nucleotide compartmentation, in Metabolic Compartmentation (Sies, H., ed) pp. 205–231, Academic Press, London
30. Mills, G. C. (1957) Hemoglobin catalysis. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. J. Biol. Chem. 229, 189–197 CrossRef
31. Sies, H., Gerstenecker, C., Menzel, H., and Flohé, L. (1972) Oxidation in the NADP system and release of GSSG from hemoglobin-free perfused rat liver during peroxidatic oxidation of glutathione by hydroperoxides. FEBS Lett. 27, 171–175 CrossRefMedline
32. Flohé, L., Benöhr, H. C., Sies, H., Waller, H. D., and Wendel, A. (eds) (1974) Glutathione, pp. 1–316, Thieme, Stuttgart, Germany
33. Sies, H., and Wendel, A. (eds) (1978) Functions of Glutathione in Liver and Kidney, pp. 1–212, Springer, Berlin
34. Bartoli, G. M., and Sies, H. (1978) Reduced and oxidized glutathione – conjugate formation from 1-chloro-2,4-dinitrobenzene and biliary metabolites in peroxisomes of the mitochondrial fraction. FEBS Lett. 219, 175–179 CrossRefMedline
35. Flohé, L., Benöhr, H. C., Sies, H., Waller, H. D., and Wendel, A. (eds) (1974) Glutathione, pp. 1–316, Thieme, Stuttgart, Germany
36. Akerboom, T. P., Bilzer, M., and Sies, H. (1982) The relationship of biliary glutathione disulfide efflux and intracellular glutathione disulfide content in perfused rat liver. J. Biol. Chem. 257, 4248–4252 CrossRefMedline
vascular function in humans. Proc. Natl. Acad. Sci. U. S. A. 103, 1024–1029 CrossRef Medline
77. Schewe, T., Schewe, T., Heiss, C., and Kelm, M. (2005) Cocoa polyphenols and inflammatory mediators. Am. J. Clin. Nutr. 81, 304S–312S CrossRef Medline
78. Schewe, T., Steffen, Y., and Sies, H. (2008) How do dietary flavanols improve vascular function? A position paper. Arch. Biochem. Biophys. 476, 102–106 CrossRef Medline
79. Wiswedel, I., Hirsch, D., Kropf, S., Gruening, M., Pfister, E., Schewe, T., and Sies, H. (2004) Flavanol-rich cocoa drink lowers plasma F(2)-isoprostane concentrations in humans. Free Radic. Biol. Med. 37, 411–421 CrossRef Medline
80. Sadik, C. D., Sies, H., and Schewe, T. (2003) Inhibition of 15-lipoxygenases by flavonoids: structure-activity relations and mode of action. Biochem. Pharmacol. 65, 773–781 CrossRef Medline
81. Arteel, G. E., Franken, S., Kappler, J., and Sies, H. (2000) Binding of selenoprotein P to heparin: characterization with surface plasmon resonance. Biol. Chem. 381, 265–268 CrossRef Medline
82. Steinbrenner, H., and Sies, H. (2009) Protection against reactive oxygen species by selenoproteins. Biochim. Biophys. Acta 1790, 1478–1485 CrossRef Medline
83. Steinbrenner, H., Speckmann, B., and Sies, H. (2013) Toward understanding success and failures in the use of selenium for cancer prevention. Antioxid. Redox Signal. 19, 181–191 CrossRef Medline
84. Steinbrenner, H., Al-Quraishi, S., Dkhil, M. A., Wunderlich, F., and Sies, H. (2015) Dietary selenium in adjuvant therapy of viral and bacterial infections. Adv. Nutr. 6, 73–82 CrossRef Medline
85. Sies, H. (1985) Oxidative stress. Introductory remarks. in Oxidative stress, (Sies, H., ed.), pp. 1–8, Academic Press, London
86. Sies, H. (1986) Biochemistry of oxidative stress. Angew. Chem. Int. Ed. Engl. 25, 1058–1071 CrossRef
87. Selje, H. (1936) A syndrome produced by diverse nocuous agents. Nature 138, 32–32 CrossRef
88. Sies, H. (1939) Strategies of antioxidant defense. Eur. J. Biochem. 215, 213–219 CrossRef Medline
89. Sies, H. (1997) Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82, 291–295 CrossRef Medline
90. Christman, M. F., Morgan, R. W., Jacobson, F. S., and Ames, B. N. (1985) Positive control of a regulon for defenses against oxidative stress and some heat-shock proteins in Salmonella typhimurium. Cell 41, 753–762 CrossRef Medline
91. Sies, H., Berndt, C., and Jones, D. P. (2017) Oxidative stress. Annu. Rev. Biochem. 86, 715–748 CrossRef Medline
92. Sies, H., and Jones, D. P. (2007) Oxidative stress. in Encyclopedia of Stress (Fink, G., ed) 2nd Ed., Vol. 3, pp. 45–48, Elsevier, Amsterdam
93. Sies, H. (2015) Oxidative stress: a concept in biology and medicine. Redox Biol. 4, 180–183 CrossRef Medline
94. Sies, H., and Jones, D. P. (2007) Oxidative stress. in Oxidative stress. in (Sies, H., ed), pp. 1–219, Academic Press, London
95. Sies, H., and Feinendegen, L. E. (2017) Radiation hormesis: the link to nanomolar hydrogen peroxide. Antioxid. Redox Signal. 27, 596–598 CrossRef Medline
96. Siegrist, J., and Sies, H. (2017) Disturbed redox homeostasis in oxidative distress: a molecular link from chronic psychosocial work stress to coronary heart disease? Circ. Res. 121, 103–105 CrossRef Medline
97. Jones, D. P., and Sies, H. (2015) The redox code. Antioxid. Redox Signal. 23, 734–746 CrossRef Medline
98. Sies, H. (2014) Role of metabolic H2O2 generation: redox signaling and oxidative stress. J. Biol. Chem. 289, 8735–8741 CrossRef Medline
99. Sies, H. (ed) (2020) Oxidative Stress: Eustress and Distress, pp. 1–844, Academic Press, London
100. Sies, H. (1980) From enzymology in vitro to physiological chemistry in vivo. Trends Biochem. Sci. 5, 182–185 CrossRef
101. Popper, K. (1976) Unended quest. Academic Press, London
102. Krebs, H. A., and Shelley, J. H. (eds) (1975) The Creative Process in Science and Medicine, pp. 1–138, Excerpta Medica, Amsterdam
103. Forman, H. J., and Toyokuni, S. (eds) (2016) Biochemistry of oxidative stress. in (Sies, H., ed.), pp. 1–219, Academic Press, London
104. Steinbrenner, H., Speckmann, B., and Sies, H. (2013) Toward understanding success and failures in the use of selenium for cancer prevention. Antioxid. Redox Signal. 19, 181–191 CrossRef Medline
105. Moss, R. W. (1988) Oxidative stress. in (Sies, H., ed), pp. 1–219, Academic Press, London
106. Sies, H. (2007) German-Japanese relationships in biochemistry: a personal perspective. Nagoya J. Med. Sci. 78, 335–347 CrossRef Medline
107. Moss, R. W. (1988) Free Radical: Albert Szent-György and the Battle over Vitamin C, pp. 1–316, Paragon House, New York
108. Sies, H. (2007) Biological redox systems and oxidative stress. Cell. Mol. Life Sci. 64, 2181–2188 CrossRef Medline