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Cellular and Molecular Life Sciences

Memories of a Senior Scientist

Biological Redox Systems and Oxidative Stress*

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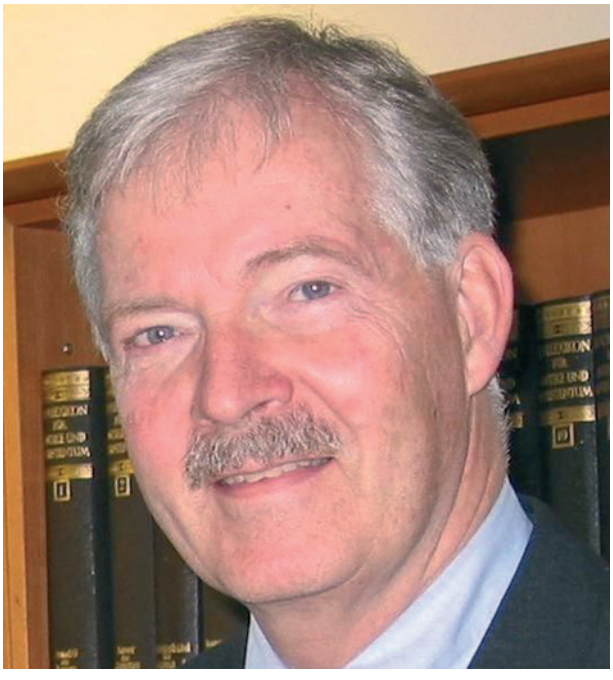

Figure 1.

Abstract. This account revolves around fascination and excitement with science. Early curiosity and fortunate opportunities can lead to a satisfying career. The privilege of performing basic research in biochemistry and molecular biology at a university coupled with teaching motivated students and working with dedicated co-workers makes for sustained thrust in the advance of knowledge. Research fields centered around cellular redox systems, oxidants and antioxidants, and the concept of oxidative stress. A noteworthy aspect is the global network of scientists joining in these endeavors worldwide.

My recent account on 'How I became a Biochemist' started out saying that, "as my own path in the world of science unfolded, I noticed the wonderful interplay of choice between a kaleidoscopic array of options and what might be called a prepared mind" [1]. The story began at Goslar, where I was born in 1942, and at the nearby city of Seesen on the Western rim of the Harz mountains in Northern Germany, where I grew up and went to school, not far from the university town of Göttingen. The first noteworthy point there was the wonderful first-grade elementary school teacher, Georg Henkel (then 28, now 86 years old and a good friend), who early on instilled enthusiasm in nature and engendered self-confidence in our own capabilities. So, one of the roots of the prepared mind was laid out early. Following a great time of adolescence and subsequent university studies at Tübingen, Paris and Munich [1], the experimental work for the M. D. dissertation (no biochemistry curriculum as yet available) was performed at Marburg and Munich under the guidance of my academic teacher, Theodor Bücher. The topic was on steady-state relaxation kinetics of the enolase reaction, one of the glycolytic reactions catalyzed with high capacity, the measurable deviation from thermodynamic equilibrium becoming a suitable measure for metabolic throughput, basically an application of simple first-order kinetics to a complex biological system [2].

* Dedicated to my wife, Nancy (Ph.D., UC Berkeley)

Postdoc Time with Theodor Bücher at Ludwig-Maximilians University, Munich

Following a two-year period in clinical medicine, notably in internal medicine with H. E. Bock at Tübingen, postdoc activities started back again at the Institute of Physiological Chemistry at the Ludwig-Maximilians-University at Munich, headed by Theodor Bücher, himself a student of Otto Warburg at the Kaiser-Wilhelm-Institut für Zellchemie in Berlin-Dahlem. Work from the outset was on biological redox systems, and "*Über die katalytischen Wirkungen der lebendigen Substanz"* by Warburg and "*Wege des Wasserstoffs in der lebendigen Organisation*" by Bücher and Klingenberg [3] were cherished reading. I was associated with Bolko Brauser, a pioneer in organ absorbance spectrophotometry, and by combining fiber optics techniques with the use of the hemoglobin-free perfused liver we were able to generate excellent signal/noise optical absorbance difference spectra from a 3–4 mm thick lobe of rat liver [4]. In combining absorbance with surface fluorescence techniques, the way was opened to noninvasive redox titration. The cytosolic NADH/NAD+ system was interrogated with stepwise changes in the lactate/pyruvate ratio, whereas the mitochondrial NADH/NAD+ system was poised and titrated by the ß-hydroxybutyrate/acetoacetate ratio, allowing direct measurement of localized redox potentials and nucleotide binding constants [5]. We also looked at the redox state of the mitochondrial cytochromes in the intact organ and the terminal oxidase of the drug metabolizing system, cytochrome P-450 [6]. The latter coincided with the early days of the drug-metabolism field, and at a workshop at Konstanz in 1968 I met Lars Ernster and Sten Orrenius from Stockholm, forming the basis of a life-long friendship. This whole research area was such a delight of congenial colleagues, including Ron Estabrook, then still at the Johnson Research Foundation at Philadelphia, and Herbert Remmer from Tübingen University, who had discovered the induction of the drug metabolizing system by phenobarbital.

My own 'niche' in the redox field opened when one evening at the basement lab in Munich I decided to look at yet another extramitochondrial pigment, the peroxisomal hemoprotein, catalase. Britton Chance had described catalase Compound I in 1947, and zeroing in on its peak in the dual-wavelength mode at 640–660 nm, I infused ethanol at low concentration as one of the hydrogen donors: gosh, it worked! This was the discovery of steady-state hydrogen peroxide production by a mammalian organ, a slightly heretical thought at the time. Discussing it with Britton Chance on one of his visits, and after appropriate control experiments and extensions, this was published in the then new FEBS Letters [7].

Research at the Johnson Research Foundation, Philadelphia

Brit invited me to join for further work at the 'JF' in Philadelphia, even together with my great technician, Annegret Marklstorfer, nee Conze (sadly deceased at the Djerba terrorist blast attack a few years ago). These were great times at the JF (see a superb account of the atmosphere then, written by Angelo Azzi whom I first met at those days there [8]), a close relationship developing with Nozomu Oshino, who had joined from Ryo Sato's lab in Osaka with his wife Reiko. Working with isolated mitochondria, Alberto Boveris, coming from Andres Stoppani's group in Buenos Aires, looked at hydrogen peroxide production under different states of respiratory activity. '*Hydrogen peroxide metabolism in mammalian organs'* was written for Physiological Reviews [9]. It took a while until we got the article together, and for the current younger reader it might be interesting to note that we did real 'cut and paste', i.e. scissors and glue, to compile the more than 550 final references together. We had about ten or even more versions going between Philadelphia, Buenos Aires and Munich, and I remember typing on my small Olivetti typewriter during summer vacation at the Etang de Berre near Marseille, while others were windsurfing. Well, the Johnson Foundation and the great personality of Britton Chance left its mark, and I am overly thankful for the good fortune of having been part of it. Longlasting friendships developed, cooperations were started, and future generations of students were fostered.

Research Group at Munich: Glutathione and Hydroperoxide Metabolism

The 'Habilitation thesis' was written in 1971, entitled '*Das Peroxysom im Hepatozyten'*, and a review article appeared in 1974 [10]. Leopold Flohé, then at Tübingen, being one of the early students in the newly established curriculum of biochemistry, was working on the enzymology of glutathione peroxidase, an enzyme yet to be fully appreciated. He had seen our papers on NADPH redox states in the perfused liver, and suggested to check whether glutathione disulfide was in fact produced at the time NADPH was oxidized, which it did. Albrecht Wendel, a doctoral student of Flohé's, joined the boat, and we had great

times along glutathione research. Two international conferences on glutathione, one at an exquisite villa at Tübingen [11], the other at the Reisensburg castle near Ulm [12] testify to this, with the Kosowers, Alton Meister and Sir Hans Krebs attending, as well as us junior people who were just starting out in research. Sir Hans, a former postdoctoral associate of Otto Warburg, after his retirement at Oxford University, at age 67 had started a whole new line of research in redox states together with Derek Williamson and Patricia Lund at the Radcliffe Infirmary, and many of his papers were close and complementary to our own interests in redox titrations. I had seen him as a student first at the 1963 Lindau Meeting of Nobel Prize Winners, and now as a young colleague met him at a workshop at Royaumont Castle near Paris in 1973 (where I also first met Joseph Tager from Amsterdam, a genuinely pleasant person and colleague), then also at the Fall Meeting of the Gesellschaft für Biologische Chemie at Düsseldorf in 1974. This was my first-ever visit to Düsseldorf, a university having just grown out of the Medical Academy. My talk was in a session chaired by Sir Hans, and the work was related to glutamine metabolism, which had been carried out largely by Dieter Häussinger, one of my first doctoral students at Munich. The talk ended, and Sir Hans, in his low voice, said that he recalls having done a similar experiment in 1935! This taught me the lesson to go back to read the literature consisting of more than just the past couple of years.

We became personal friends, paying mutual visits to him and his charming wife Margaret at Oxford (including a punting spree) and to Munich (I vividly remember a party in our small apartment with Feodor Lynen and Otto Wieland concentrating on a bottle of Chivas Regal), and having long walks and discussions at the Aberdovey beaches in Wales or in the Alps. His wonderful sense of humor is most memorable, Sir Hans having been a most unassuming personality, always curious to find out something new; a wonderful experience.

Work was going well, with great young medical students joining. One of my best early students, as already mentioned, was Dieter Häussinger. This is how I recruited him: in a written exam for the second-year medical students, he had finished way before everyone else and started smoking his pipe, so we got into talking… The first postdocs from abroad joined the team: Karen Moss from Brian Chappell's group in Bristol was the first Humboldt Fellow of many to come, Theo Akerboom joined from Tager's group in Amsterdam, and there was a flurry of international meetings (at one stage in the cytochrome P-450 string of meetings, Judd Coon from Ann Arbor sighed: "I've got to fly home to pick up some more slides…"). Ingrid Linke was the core technician, keeping things running when I was out of town. We also had exciting Fasching (Carnival) feasts in the spring, sometimes even too exciting.

At Munich, interest in oxygen-related topics flourished, and on one memorable day in 1977 we founded the Munich Oxygen Club in the Max-Emanuel-Brewery, a group of colleagues that made their name in the field in the decades to come: Erich Elstner, Manfred Saran, Wolf Bors, Hans Nohl, Peter Eyer and many others.

The main research support came from the Deutsche Forschungsgemeinschaft, Bücher had taken initiative to found the Sonderforschungsbereich 51, entitled "*Biochemie und Molekularbiologie in München*", including members from university (Feodor Lynen) and from the Max-Planck-Institute as well as the Diabetes Institute in Schwabing (Otto Wieland). Young researchers got their first independent projects and profited from interdisciplinary cross-fertilization (one of them, for example, was Robert Huber, Nobel Prize 1988 for chemistry). A highlight was the annual retreat to Lake Tegernsee near the Alps, called '*Symposium Gentianum*', named appropriately for the location at the place where *Enzian Schnaps* was (legally) distilled.

Düsseldorf University: The Institute of Physiological Chemistry

As it goes in German academia, a time comes to wander off: I was appointed Full Professor (*Ordinarius*) at the Department of Physiological Chemistry I at Düsseldorf, starting in the fall of 1979. Wolfgang Staib, chair of the Department of Physiological Chemistry II, had things covered well regarding faculty matters and teaching arrangements, and he gave valuable administrative advice, so luckily I could concentrate on building a research group more or less from scratch with almost no eclipse from the move. One first area was redox cycling and thiol redox state, working with Regina Brigelius, and there was an early review on quinone redox cycling together with Hermann Kappus [13]. Enrique Cadenas from Buenos Aires had joined as Humboldt Fellow, and with Armin Müller and Peter Graf we looked at a new selenoorganic compound, carrying the order number (*Prüfzahl*) PZ 51, brought to us by Erich Graf and Mike Parnham from the Nattermann company at nearby Cologne. This compound, later known as Ebselen, exhibited glutathione peroxidase-like activity, and we shared this whole topic with Albrecht Wendel's group in our joint glutathione-oriented research, resulting in several papers published in Biochemical Pharmacology

(our first one, [14]). Low-level (ultraweak) chemiluminescence detected by red-sensitive photomultipliers or later on by a Germanium diode detecting in the near-infrared at 1270 nm, was a means to study singlet oxygen chemistry and biochemistry. This noninvasive technique allowed us to obtain novel insight into the biology of electronically excited states. It was a great pleasure to interact with Giuseppe Cilento and his group from Sao Paulo and with Waldemar Adam from Würzburg on electronically excited triplet carbonyls. Many co-workers from the Institute at Düsseldorf and visitors from abroad made this a very lively time, including Herbert de Groot and Thomas Noll, specializing on hypoxia and lipid peroxidation, Heribert Wefers on menadione redox cycling, Theo Akerboom on glutathione disulfide and glutathione S-conjugate transport, and Gianna Bartoli from Rome on glutathione (GSH) release, José Estrela from Valencia on calcium transients during drug metabolism, Denis Crane from Brisbane on coenzyme A mixed disulfides, Gail Gurtner from Baltimore on pulmonary vasoconstriction by arachidonate metabolites, Francisco Romero from Valencia on glutathione compartmentation, Toshihisa Ishikawa from Sapporo on cardiac glutathione S-conjugate transport, Raj Sohal from Dallas on pentane production and aging in the housefly, Dieter Häussinger then from Freiburg on pyruvate dehydrogenase and on glutamine metabolism, Jim Kehrer from Austin on ischemia/reperfusion, to name a few. Peter Graf was an excellent technician in many of these projects.

National Foundation for Cancer Research (NFCR)

At these bustling times, on the other side of the Atlantic an exciting organization had come into being: in 1973, Franklin Salisbury, a Washington-based lawyer, and his wife Tamara had founded what was known to be the National Foundation for Cancer Research, NFCR. The co-founder was Nobel Laureate Albert Szent-György, based at Woods Hole, Massachusetts. A major reason for establishing NFCR was to provide research funds to enable Szent-György and scientists from all over the world to do basic research in the cancer field, the motto being '*Laboratory without Walls*'. It was an extremely fortunate event that I was invited to present our research at the 1983 NFCR meeting at Montecito, California. Many of those present (Fig. 2) have made fundamental contributions in the decades to come. Later that year, Franklin and Tamara had a small meeting at Braunlage in the Harz Mountains in Germany, and I flew a small Cessna plane to a nearby grass airstrip to join there for presenting our results. Whether it was the fact of flying the plane as a private pilot or whether it were our research results: Tamara and Franklin were impressed (a scientific advisory board was around as well, by the way), and I was asked to join NFCR as a Project Director, being funded generously for basic research, starting from 1984, from 2000 onwards as an NFCR Fellow, now under the leadership of Franklin Salisbury, Jr. and Sujuan Ba. This wonderful instrument of research support, based on initial trust, and with very little bureaucratic strings attached, certainly has contributed enormously to productivity in our group. This is an appropriate place to express my sincere gratitude.

Society for Free Radical Research (SFRR)

Trevor Slater from Brunel Unversity at Uxbridge, United Kingdom, and his associate Robin Willson were instrumental in founding the Society for Free Radical Research in the U.K. in 1982 (Fig.2). Trevor linked up with Hermann Esterbauer from Graz, Austria, and Mario Dianzani from Torino, Italy, and Mario Comporti from Siena, Italy. Being part of this group, I enjoyed the scientific colleagiality and open interchanges in these early days. This wonderful spirit filtered down through the whole field worldwide, and still today the research field on free radicals and related topics is characterized by a friendly and generous atmosphere.

The Third Biennial Meeting of SFRR was held at Düsseldorf in 1986, a highlight in the field as well as for us. I had just published the book "Oxidative Stress", with the definition of this term and of the concept in the Introductory Chapter [15], and a larger article on the biochemistry of oxidative stress [16].

Oxygen Club of California (OCC)

Lester Packer (Fig. 2), from the Department of Physiology at UC Berkeley, had also been at the JF with Britton Chance, and his extraordinary personality and enthusiasm to start new research made his group a center in free radical research, based methodologically on electron spin resonance techniques, utilized by John Maguire, Rolf Mehlhorn and Alexandre Quintanilha. NFCR had a superb meeting at Berkeley, topped by a boat ride in San Francisco Bay. I remember vividly the radiant Tamara Salisbury and the scientific director, ex-Surgeon General of the army, Charles Pixley, stepping off the boat after a great party. The Packer lab also was active in teaching, in particular a well-attended class on aging, together with a neuroendocrinologist, Paola Timiras. In 1984/

Figure 2. National Foundation for Cancer Research (NFCR) Research Conference at Montecito, CA, 11–12 February 1983. Front row (from left): Harold Schwartz, Lester Packer, Franklin Salisbury, Albert Szent-György, Trevor Slater, Patrick Riley, Hermann Esterbauer. Second row (from left): Keith Ingold, Bill Pryor, John Ward, Rolf Mehlhorn, Helmut Sies, Alexandre Quintanilha, Norman Krinsky, Peter Gascoyne, Bruce Demple, Martyn Smith, Robin Willson.

85, I took a sabbatical with Bruce Ames at the Department of Biochemistry at UC Berkeley in Barker Hall, closely working together also with Lester Packer and Martyn Smith. My work was on the OxyR regulon, and we noticed that deletions of this regulon led to enormously augmented spontaneous mutations, whereas strains overexpressing OxyR had considerably less mutations than controls.

These days laid the foundation for much of the subsequent development: Lester Packer founded what is now known as the 'Oxygen Club of California (OCC)', www.oxyclubcalifornia.org, cherished for its great Biennial World Congress, held at the Fess Parker Doubletree Hotel at Santa Barbara. These meetings also extended to other sites, e.g. to Cadiz, Spain, and Alba, Italy, as well as to Portland, Oregon, in joint meetings with the Linus Pauling Institute headed by Balz Frei, a former associate of Bruce Ames. Not enough: Gordon Conferences were inaugurated, on Oxygen Radicals, on Nitric Oxide, and on Carotenoids, with Lester Packer and Norman Krinsky (Fig. 2) being in the forefront. Many of these meetings were sponsored by NIH and other government agencies, by Unesco, WHO etc., but to a considerable degree also by industry. Genuine scientific interest was expressed by colleagues coming from industry, illustrated by the fact that several of the Lifetime Members of OCC are from industry, for example Manfred Dunker from the Henkel Company.

Singlet Molecular Oxygen

Singlet oxygen as an electronically excited state is important not only in photooxidations but also in chemiexcitation, i.e. excitation without light energy. We used the thermodecomposition of an endoperoxide to generate singlet molecular oxygen at a controlled rate, enabling us to study singlet oxygen chemistry in biological settings. Paolo di Mascio in his dissertation work studied the reaction of the endoperoxide of 3,3'(1,4-naphthylidene) dipropionate ($NDPO_2$) in detail, examining the dimol photoemission at 634 nm and 703 nm as well as the monomol emission at 1270 nm [17]. We looked at DNA single-strand breaks in plasmids in work by Heribert Wefers, together with Dietrich Schulte-Frohlinde from the Max-Planck-Institute for Radiation Chemistry at

Mülheim. With Steen Steenken from the same Institute, Wolfgang Schulz and with Paul Devasagayam, visiting from the Babha Atomic Research Institute at Mumbai, India, the molecular aspects were studied in a detailed fashion. Likewise, mutagenicity of singlet oxygen was studied in a mammalian SV40-based shuttle vector in joint work with Carlos Menck from Campinas, Brazil, and Alain Sarasin from Paris.

Carotenoids: Lycopene

Unlike the enzymatic defense against hydroperoxides as effected by catalase and GSH peroxidases, there is no known enzymatic defense against the short-lived singlet oxygen. Linus Pauling in the 1930's had noted a role of carotenoids in singlet oxygen quenching, utilizing the extended system of conjugated double-bonds to dissipate the energy to the solvent as thermal energy, leaving the molecule intact. Thus, carotenoids would serve like an enzymatic catalyst. Interested in biological defense against singlet oxygen, we examined various classes of compounds and found that lycopene, the red pigment occurring in the tomato, was the most efficient singlet oxygen quencher, with the second-order rate constant being near the diffusion limit [18].

Wihelm (Willi) Stahl joined the group in 1990, establishing carotenoid analysis, the basis for a long-term development. Specific patterns of cis-trans isomers of lycopene and ß-carotene in human serum and tissues were described, and first steps into the field of human nutrition were taken: Uptake of lycopene was found to be greater from processed than from unprocessed tomato juice or from tomato paste than from fresh tomatoes. Lycopene came to be a biologically important carotenoid for the human. Biokinetics, the application of pharmacokinetic principles to micronutrients, vitamins and trace elements, is important in assessing the function of compounds occurring in minute amounts.

Carotenoids and their stimulatory effect on gap-junctional intercellular communication was a topic of interest. 4-Oxoretinoic acid generated from canthaxanthin was stimulatory, as were a number of synthetic carotenoids. Synergistic antioxidative effects of lycopene and lutein were observed in membranes, and divergent optimum levels of lycopene, ß-carotene and lutein in cells were identified. We consider the latter observation of general interest with regard to the large human antioxidant trials, noting that those employing higher doses in the human intervention studies provided rather negative results, contrary to expectations: Paracelsus was right! Dietary tomato paste was found to protect against ultraviolet light-induced erythema in humans, and with Willi Stahl several studies on the concept of nutritional protection against skin damage from sunlight were performed (see [19]).

Peroxynitrite, Nitric Oxide, Selenium

The biology of peroxynitrite, a product of nitric oxide and superoxide, became a research topic after Joe Beckman's pioneering paper. We noted that ebselen, the GSH peroxidase mimic, reacted with peroxynitrite, in studies with Hiroshi Masumoto from Tokyo and Wim Koppenol from Zurich. Karlis Briviba and two other young colleagues from the former Soviet Union, Victor Sharov and Ivan Roussyn, embarked on various aspects of defense against peroxynitrite. Not only ebselen, but also glutathione peroxidase protected against peroxynitrite, exhibiting peroxynitrite reductase activity. Other selenoenzymes such as selenoprotein P or thioredoxin reductase as well as tellurium compounds displayed this activity, with Gavin Arteel from Chapel Hill and the doctoral students Darius Buchczyk and Ronald Tamler being instrumental in these studies. The field of selenium compounds developed rapidly, as reviewed together with a Humboldt fellow from Bangalore, Govind Mugesh, and in joint work with Claus Jacob these topics were extended into protein chemistry. Annika Assmann found that selenomethionine oxide was readily repaired by glutathione, opening the attractive strategy of channeling protein oxidation to a readily repairable site, selenomethionine, thereby protecting less well repairable aminoacid sidechains in proteins. A new field opened with emerging knowledge on signaling cascades. Stefan Schieke demonstrated that activation patterns of mitogen-activated protein kinases elecited by peroxynitrite were attenuated by selenium supplementation of cells in culture.

Signaling, Aging Research

Beate Meier made the pioneering observation that human fibroblasts release reactive oxygen species in response to interleukin-1 or tumor necrosis factor-alpha [20] and in response to treatment with synovial fluid from patients suffering from arthritis. These observations provided a bridge between biochemistry and immunology, which received considerable attention subsequently.

Lars-Oliver (Ole) Klotz and his team in the lab dissected the pathways of quinone-induced signaling, which involves the epidermal growth factor receptor as a common mediator [21]. Peter Brenneisen and his

group examined factors generated and released by tumor cells, acting on gap junctional communication between human fibroblasts. Klaus-Dietrich Kröncke analyzed the role of nitric oxide in zinc-finger-dependent transcription. Thus, the exciting emerging aspects of signaling pathways and mechanisms were addressed.

Cristina Polidori from Perugia, Italy, joined for work on the topic of aging research, having access to centenarians. The basis for identifying profiles of antioxidants in human plasma was worked out [22]. Aging research has advanced in several directions. We worked on human skin, particularly on the effects of ultraviolet radiation, in joint work with Jean Krutmann, in the framework of a core grant (SFB) of the Deutsche Forschungsgemeinschaft, "Molecular and cellular mediators of exogenous noxae", initiated together with the chair of dermatology, Thomas Ruzicka, in 1995.

Flavonoids: Cocoa Flavanols and Vascular Responses

In 1998, the Institute of Medicine of the National Academies held a meeting of the Panel on Dietary Antioxidants (Chair, Norman Krinsky) at Washington, D.C. at which I gave a survey lecture. In the audience there was Harold Schmitz, working at the Hackettstown, New Jersey, facility of Mars, Inc., who had recently embarked on identifying procyanidins and flavonoids in cocoa. We discussed cocoa polyphenols and research on dietary antioxidants: the beginning of a longterm research relationship. Gavin Arteel quickly found that cocoa polyphenol oligomers were effective in protecting against peroxynitrite [23]. (-)-Epicatechin, the flavanol monomer building block of the procyanidins in cocoa, thus became a focus of interest in our research group. Peet Schroeder found that protection by epicatechin was particularly efficient for nitration reactions as compared to oxidation reactions.

Tankred Schewe, a colleague established in lipoxygenase research, joined the group, and he found that polyphenols from cocoa inhibit mammalian lipoxygenases at concentrations of physiological interest [24]. The efficiency of epicatechin in protection against peroxynitrite further directed our focus to the vascular endothelium, and it was fortunate that Malte Kelm at the Düsseldorf cardiology clinic, an expert on vascular endothelial function with emphasis in nitric oxide research, agreed to join in: the doctoral student Christian Heiss employed the noninvasive technique of measuring flow-mediated dilation of the brachial artery, establishing that cocoa flavanols exhibit vascular responses concomitant with increased concentrations of circulating bioavailable plasma nitric oxide [25]. Interestingly, the vascular effects apply not only to conduit arteries but also to microcirculation as revealed in a noninvasive study on human skin.

What makes you 'senior'?

The wonderful essence of life as a university researcher and teacher is that there is always the exciting next question and, fortunately, there is the enthusiastic next young student taking up what previous research had unraveled, eager to comprehend the problem and the techniques to solve the problem. It is a great privilege to be part of this, and to be supported by funds from trustful taxpayers and other sponsors. One finds that peers spend their time reviewing the work for publication, and that there are colleagues going through the tedious task of arranging scientific meetings which allow direct exchange of results and ideas long before being published in the scientific journals, and that there are well-known researchers taking their time to help decide on grant applications or fellowship awards. This is all fine, and one can indulge in the benefits of these services in the scientific community. Then, there comes the time of paying back: the Deutsche Forschungsgemeinschaft asks for help reviewing grant applications and to take part in site-visits for larger grants, the Alexander-von-Humboldt Foundation asks for service as a member of the Selection Committee to help identify top talent from around the world for their fellowships, industry asks for advice in their research programs, organizers of meetings ask for overview talks or educational lectures (as apart from truly research-oriented lectures), universities ask to help found core facilities or to take part in committee work, and the like. Thus, an unsuspecting dedicated researcher is exposed to a gradual, creeping demand for help, which is not directly addressing the focus of research but reaches out somewhat further. Is this what might define 'becoming senior'?

Maybe there could be another angle, slightly more biological: a senior scientist could, like a senior citizen, be defined as one of grandfather status: scientific offspring of one's own students is a highly satisfactory mark of making one senior. In my case, I was very fortunate to see several of my former students shape their own careers in science in a highly successful way, generating many 'scientific grandchildren'.

Helping organizations flourish is another aspect of seniority. In my case, it was the Northrhine-Westphalian Academy of Sciences, to which I was elected in 1991. Having been active in the 'Class of Natural

Sciences and Medicine' in several types of activity, from 2002 I served four years as President of that Academy and from 2003 onwards as Vice-President of the Union of German Academies of Science. From 2005 onwards, I joined as Member of the Council of the Nobel Prize Winners Meeting at Lindau, serving the most pleasurable task of facilitating interaction of top young talent from around the world with the Nobel Laureates.

I close this brief rendition with one thought: the worldwide network of science and its productivity is based on a network of individuals, and it is the interaction of these scientists and their enthusiasm which generates the future development. I would like to mention one close personal friend and mentor who had much to do with my own path in the world of science: Gustav V. R. Born, Department of Pharmacology at King's College, London, and later at the William Harvey Research Institute. Gustav can be called truly senior as scientist, having helped shape scientific careers in all parts of the world, including many in Germany, with myself being lucky to know this superbly generous scientist.

The motto I give to new students joining the lab: "Science is to learn to live with a constant level of frustration *and* enjoy the fascination!"

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