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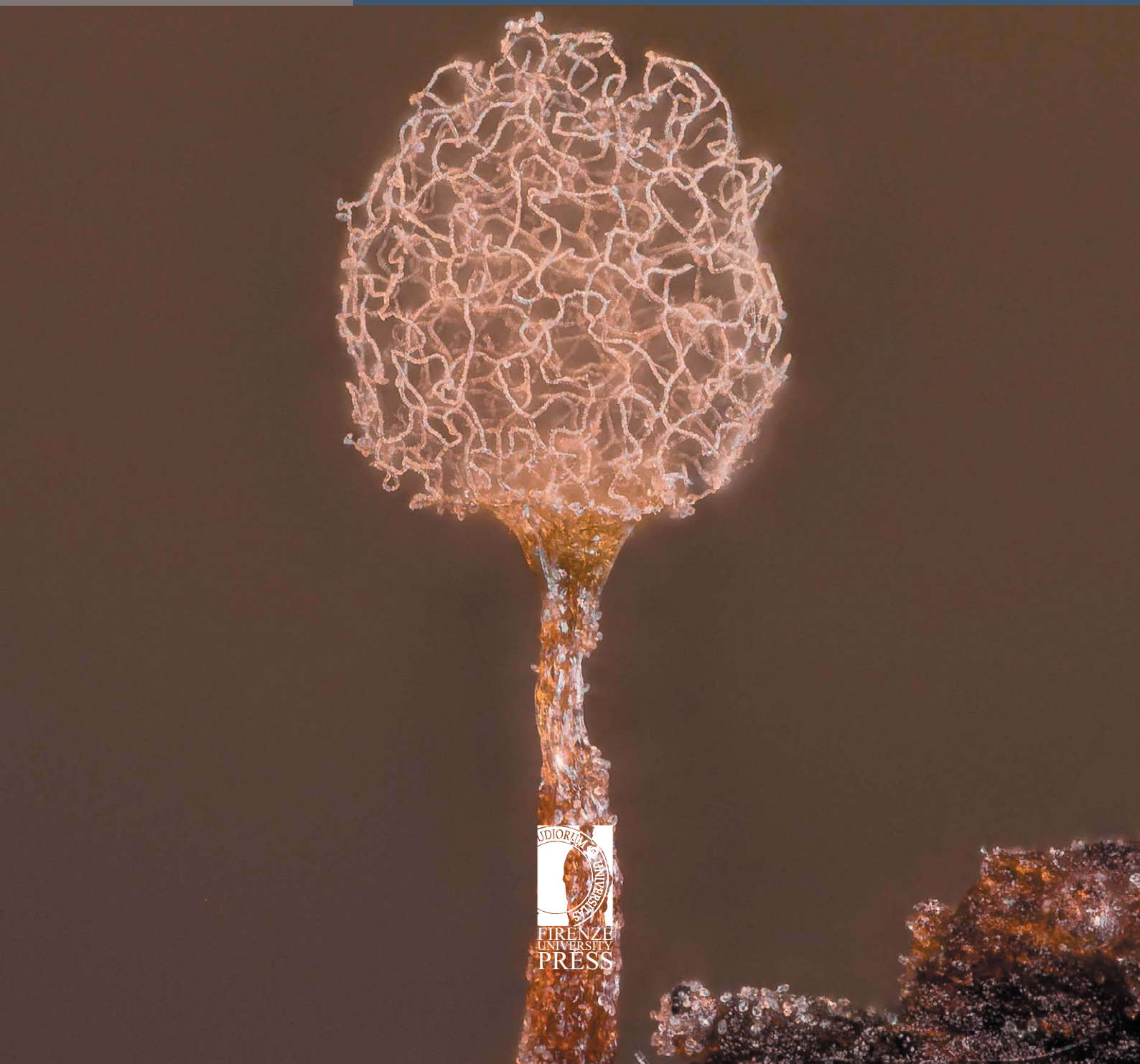

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To Print or not to Print?

Preprints and publication: how the Covid-19 pandemic affected the quality of scientific production

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An interesting paper recently published in *Peer J.* by Enrique Teran and coworkers casts light on a peculiar side effect of the Covid-19 pandemic that concerns the quality of articles that appeared as preprints in archives or as regular papers in peer-reviewed scholarly journals.[1]

The authors report a detailed perusal of the scientific publications related to research on Covid-19 in a portion of the year 2020.

What emerges from the study is that over the total number of preprints uploaded in the archives' servers, that are not subjected to a formal peer-review process, only about 5.7% were later converted into regular articles and published in scholarly journals after a regular peer-review process. The statistics is based on a global sample of 5,061 preprints uploaded in three different archives.

The fast, almost immediate dissemination of experimental studies has certainly played an important role during the pandemic. In fact it was promoted by the International Committee of Medical Journal Editors.[2] Moreover, the World Health Organization[3] and some journals require that manuscripts be shared as preprints before being sent to reviewers. In this way preprints are exposed to a public form of peer-review in real time.

Fast publication as preprints certainly helped different research groups to exchange data and interpretations, hypothesis and proposals for public health guidelines to secure a powerful response to the virus attacks.

However, a conversion of 5.7% from preprints to regular articles seems to be too low. This may be due to the emergence of more accurate studies, to repeated verifications of the presented data on different samples, and to the very fast communications of results that made fresh data look like old and outdated findings.

But on the other hand, Covid-19 related topics are very delicate matters for their social and political consequences. No doubt they must be treated with extra care. So many fake news, accusations of conspiracies, distrust in official authorities' statements have filled newspapers and websites, including the social networks that in this specific case have probably shown the dreadful and terrific power of some people's madness and ignorance.

Peer-review requires time. Reviewers are often reminded and urged to return their comments within few days. We know that a "short lapse between submission and acceptance" is a very appreciated feature of scholarly journals. Authors are anxious to see their work published, and sometimes do not appreciate enough the benefit of an accurate review.

Teran's article also reports that between February and May 2020 about 17,500 articles have been published in peer-review journals indexed in PubMed, suggesting that even during the Covid-19 emergency, scholarly journals kept pace with an enormous pressure from the scientific community and publish their articles after a formal peer-review process.[1]

Another interesting point is that articles published in scholarly journals received more attention and a higher citation rate than preprints. The citation count is one of the quantitative indicators of the scientific relevance of a publication (although not all citations may be positive).[4]

In conclusion, while the scientific communities have all the tools to confirm or reject the findings published in open access preprints, the same does not hold

for average non-specialized readers that sometimes take advantage of partial or limited results to discredit the conclusions of serious studies.

And in our democracies the recent events have proved that these differences between scientists and self-proclaimed "experts" do matter.

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Feature Articles

Faraday's Dogma

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Abstract. Contemporary scientific research is competitive, costly and coupled to the parallel universe of commerce. A Faustian bargain between scientists and politicians allows the funding to flow. There is another path: to slow down, think and experiment without the pressure of competition and frequent publication. That path will come at a cost: reduced funding for people and equipment. The article compares and contrasts the most creative musical and visual artists with the current scientific model. I suggest that science requires acceptance that true creativity can only come by decoupling from current commercial and political imperatives.

Keywords: science and art; scientific creativity.

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When asked the secret of doing science, the great chemist Michael Faraday, replied in the early 1800s

> Work, finish, publish

- no-nonsense common sense from a humble autodidact, who rose from working class obscurity to being offered (and refusing) the Presidency of the Royal Society in his maturity. Faraday's extraordinary experiments exploring electromagnetism are legion. Given his status, his words are treasured to this day by scientists. Surely, those words have been drummed into countless young postgraduate researchers by well-meaning seniors for generations. His vision of experimental science remains de rigeur to this day:

> I am no poet, but if you think for yourselves, as I proceed, the facts will form a poem in your minds.

That quote hints that science may be an ethereal enterprise, less focussed than a take-no-prisoners voyage of discovery. Science is poetry? Alternatively, Faraday's insistence on "facts" as the stuff of his poetry is to many reassuringly grounding, bringing the practice back to earth. The brevity and subliminal appeal to common-sense of both quotes seems to me a hallmark of British science. I see a direct line, for example, from no-nonsense Faraday to (in my mind) the most British scientist of all, the (New Zealander!) Ernest Rutherford, who classified all science as physics or stamp-collecting, and

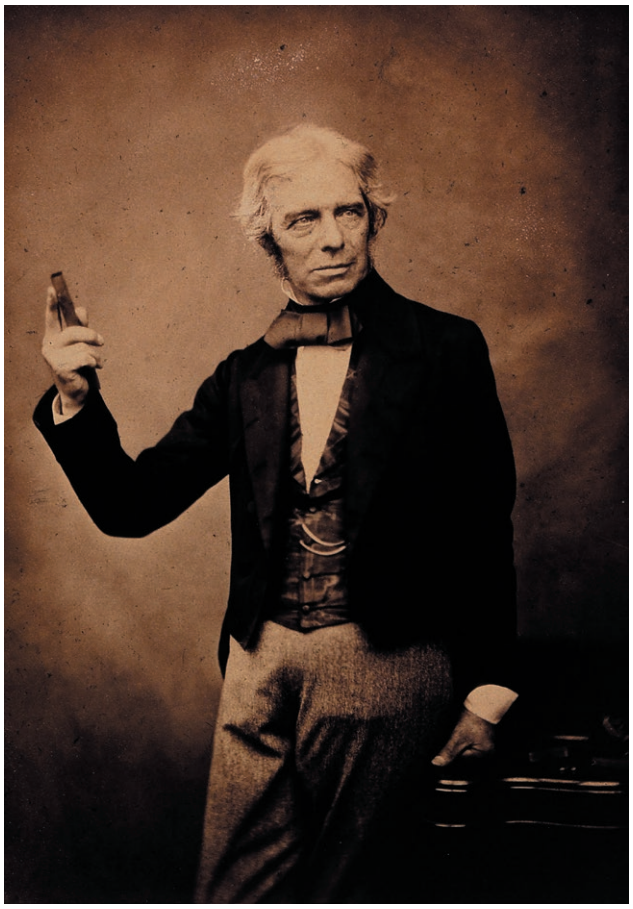

Figure 1. Michael Faraday posing with a tool of trade (a magnet?). Photograph by Maull & Polyblank. Wikimedia Commons. Photo from the Wellcome Library (ICV No 26801). Photo number: V0026348.

reckoned the odds of betting against science at 10^{12} to 1. So it came as a surprise to me to come across the following words, also from Rutherford…

> I think a strong claim can be made that the process of scientific discovery may be regarded as a form of art. This is best seen in the theoretical aspects of Physical Science. The mathematical theorist builds up on certain assumptions and according to well understood logical rules, step by step, a stately edifice, while his imaginative power brings out clearly the hidden relations between its parts. A well constructed theory is in some respects undoubtedly an artistic production. A fine example is the famous Kinetic Theory of Maxwell. … The theory of relativity by Einstein, quite apart from any question of its validity, cannot but be regarded as a magnificent work of art.

Those words can be parsed to imply that creativity resides in science, just as in art. That claim is no threat to modern science, but what if it were taken more literally? It is somewhat startling to a card-carrying scientist (including this one) to read Rutherford's words once more..."scientific discovery may be regarded as a form of art"! Is science another domain of artistic practice, alongside sculpture, film-making, etc.? That reading is perhaps overblown; after all, Rutherford explicitly invokes the notion of a logical progression to scientific "discovery", an implicit credo dear to the hearts of many practising scientists. Yet he also allows for the creation of scientific theories as works of pure art, regardless of their validity. Hmmm. Do scientists "create" rather than "discover"? And, for that matter, do artists "discover" or "create"?

These conundrums are age-old, but largely forgotten in the day-to-day hustle and busyness of the massive production line of science, whether from a crowded lab in some unprepossessing rural university, or the fabled CERN scientific complex, so extensive that its "lab" sprawls across a national border, straddling France and Switzerland. Perhaps they are forgotten for a simple reason: science is expensive. In fact, science is far more costly than even the most costly art productions, including the bloated budgets of Hollywood productions. After all, Hollywood films are deemed to have failed unless they recoup their production costs, and (far) more. Value is no more, or less, than a balance of expenditure over costs. Ask a film producer, or a crusading journalist, or the imaginary taxpayer, summoned into the mind of any politician as he or she weighs up a country's annual Budget. Science too is likewise constrained. Its triumphs, such as the extraordinarily rapid development of Covid vaccinations are sure indicators of its value. Likewise, the current crowd of "scientific experts" quizzed by the media on the Covid epidemic: a daily parade of epidemiologists from all corners, whose variety of models will surely explain any eventuality. Despite the public swagger of science, it remains at heart a fragile construction. Even at its most strident, its "facts" are unclear. To give one current example, the debates over effective quarantine measures in Australia gloss over the "fact" that aspects of the fundamental science of viral transmission, from fluid mechanics[4] to soft-matter science[7], remain unknown. Despite an apparent consensus, science remains a human activity, far more complex than a well-equipped voyage of discovery. Any reckoning of the (financial) value of science is messy and ultimately hopeless. The conscientious accountant must include the price of microplastics in the environment, of fossil fuel extraction, as well as the benefits of vaccinations.

It seems to me that in the midst of Covid and the apparent triumph of scientific research, we would do

Figure 2. (L to R:) Models for *Florentia* (2006). Robert Owen. *Cadence no. 1 (a short span of time)*, 2003.

well to sit quietly, and reflect on the the nature of science itself. My own thoughts were triggered by a recent visit to the Heide Museum of Modern Art in Melbourne, where I spent a few hours at a retrospective exhibition of the Australian modernist Robert Owen, "Blue Over Time", featuring sculptures, paintings, and assorted other pieces[5].

Owen is a deep thinker and profound artist, whom I have been fortunate to occasionally spend time with. (A recommended introduction to his work is the beautifully produced recent monograph "Robert Owen - A Book of Encounters"[6].) He's worth knowing for his own sake: he spent a few years in the early 1960s on the Greek island of Hydra together with an extraordinary community of writers, musicians and scholars, including Leonard Cohen and (lesser known globally, but equally influential to Australians) Charmian Clift and George Johnston. The flavour of that community is beautifully captured by a couple of exhibits at Heide: a pair of reed flutes, hand-crafted by Owen and exchanged with Cohen. In return, Owen was given a copy of Cohen's latest book of poetry, "Flowers for Hitler" (1964), inscribed with the poet's dedication:

> For Bob
> like your reeds there
> is a special way to blow on these
> All good things
> Leonard,
> Hydra,
> Winter 1965

The practical advice of Faraday and the dreamy lyrics of Cohen are so different, both in intent and content, that visiting aliens could be excused were they to suggest the existence of multiple distinct life-forms on earth. It seems that scientists and artists have evolved entirely distinct ways of thinking and doing on the same planet. Or perhaps Faraday, Maxwell, Einstein, even Rutherford, *were* poets, like Cohen?

On the other hand, my Heide visit rekindled the converse thought: perhaps (good) artists are in fact scientists? I find Owen's works scientific as well as artistic. So much so that after being shown his sculptures a few years ago, I began researching the concept of "tangled polyhedra" from the perspective of topology and graph theory, a subject which continues to preoccupy me and other scientific colleagues, more than a decade later. (Owen and I described those connections elsewhere[3].) My interest was triggered by nothing more (or less) than Owen's artworks, yet I – in all honesty, dishonestly – continue to justify my research on these tangled forms in technical papers by its relevance to things of "value", such as interwoven molecular frameworks in modern synthetic materials. The "value" of that field of fundamental scientific research is in turn routinely justified to the broader science community (in less specialised science publications and grant applications) via its relevance to *really useful stuff*, such as "designer materials" tuned to store hydrogen for green energy, or capture carbon dioxide to mitigate global warming. All scientists know the game well, for those who will not or cannot play rarely survive beyond their first post-doctoral post or grant round. One more confession: (nearly?) all the scientists I know admit to the essential dishonesty of that game, playing along to keep the funds and publications flowing. The game is an ingrained aspect of contemporary science, tolerated as an obligatory distraction from the real business of scientific research. But just what is that real business?

It may come a surprise to many non-scientists to learn that the deeper one looks into the practice of scientific research, the closer the practice of fundamental science resembles the art of Owen and Cohen than the well-paved path from the lab to the publisher, chronicled by Faraday. Faraday claimed his "poems" were built with "facts". And so they were: his magnets and galvanometers did not lie. Neither do the reams of data thrown up by CERN's accelerator, or the the microwave map of the sky. But the spinning and weaving of that data into a scientific fabric, offered to the otherwise uneducated masses as the signature of the elusive Higgs particle, or an echo of the Big Bang reverberating around the cosmos, is neither a truth nor a lie. It is a story, spun by human creativity. There is no single road from "work" to "publish". Conversely, it is not too far-fetched to associate visual art, or literature, with "facts", though those facts are not readily detected by a scientific instrument. Conjunctions of colours, which lie at the heart of Owen's abstract pixellated paintings, and words, which fizz within Cohen's lyrics, catalyse common, unquantifiable, emotional responses in very different people.

In the current climate of usefulness and value, the intrinsic slipperiness of science, as opposed to the incremental and essentially phenomenological nature of engineering, is too often denied. Art is an essential reminder. Artists explicitly embrace the irrational: the essential source of creative ideas. We scientists, on the other hand, flee from such flakiness, preferring to shelter within more politically-acceptable quarters, whose admission price is alignment with the prevailing notion (not notions) of "value": economic growth. Our adherence to the "value" of science, measured and funded in terms of economic value, has cost us dearly. I was reminded of that cost reading an essay by the eminent quantum theorist, David Bohm, "On the relationships of art and science" (available in a collection of essays by Bohm, "On Creativity"[1]), recommended to me by Owen. In his essay, Bohm discusses the new artistic languages in the twentieth century, from Cézanne to Cubism, Constructivism and Mondrian and their common feature: the emergence of an entirely new form of art, which involves new structural elements which in themselves have no meaning, but which combine to form structures whose meanings arise from the imagination of the artist alone. The value of such art, Bohm argued, lies in its possibility to shed light on how structure itself is perceived by the senses, potentially opening new ways of seeing the external environment. The artist who wrestles with forms, reveals, at his or her best, "new general understanding of structure at the perceptual level… From this, the scientist can form new abstract ideas of space, time and the organization of matter." His argument can be interpreted as one of peripheral relevance to most scientific researchers: after all, how many of us are looking at new ideas of space or time? That response is comforting, but too easy. For surely all physicists, chemists and molecular biologists are looking deeply into "the organization of matter"?

Like Rutherford's comparison of scientific theory with art, Bohm's argument seems astonishing today. Both quotes betray a proud indifference to the conventional practice of scientific research, articulated so clearly by Faraday. In contrast, today's science can be described as Faraday-lite: obsessed with continuous reinforcement of the fiction of "values", thereby sidestepping deeper sources of thought.

Can scientific culture value deep thinking? Thankfully, yes. Witness, for example the recognition of its importance by Rutherford and Bohm (and numerous other "great" scientists, including Einstein, Poincaré,…). If so, *how* can scientists recover the practice of deep thinking? First, we must abandon current obsessions guiding "good science". The fiction that scientists work better in a hurry must be laid to rest, with apologies to Mr. Faraday. Like Slow Food, whose origins lay in rejection of fast food by Italian consumers and growers, Slow Science is healthy. Second, the cult of collaboration must be exposed for what it is: an empty belief. Though group-research is important to some, larger groups are also prone to group-think mediocrity – science by committee – rather than creative ideas. Worse, the very idea of a scientist working alone remains acceptable in mathematics, but anathema in the other sciences. Perhaps, most painfully but most importantly, we must abandon the credo that binds us to the political and sociological "system" whose measures of value allow only short- to medium-term productivity and utility. That will happen anyway, it's called engineering. (At this point, I hear murmurs of disapproval from many quarters: acceptance of a basic distinction between science and engineering is an implicit nod to elitism. In fact, both practices require their own elites.) Like artists, scientists must accept that true creativity is unlikely to be funded to the level required to sustain another CERN or a (wo)manned mission to Mars, even with Elon Musk's purse.

More to the point, the time has surely come for science to definitively break the yoke that binds science to "value". Within my own time in science since the 1980's, I have seen the wholesale reassignment of scientists as de-facto engineers. Giant industrial labs, such as IBM and Xerox in the US, have disappeared, their (engineering) research now funded by the taxpayer, being done in physics and chemistry labs in academia. More recently, Covid has induced extreme financial hardship on universities in Australia, dependent on fee-paying by non-Australian students to sustain a large body of self-identifying science researchers, all claiming to conduct ground-breaking and world-beating (and expensive) research. Sadly, it is not too far-fetched to argue that unfettered scientific research is all but gone, except in the most privileged of academies around the world. If it is to revive, perhaps reversion to fewer, modestly-funded (dare I say "elite"!) scientists is essential in any case.

I was reminded of these uncomfortable prescriptions for the future of science most recently, and forcefully, by a fascinating article by the Genoese art critic Germano Celant, first published in 1967. Celant's article is devoted to the philosophy of a group of Italian artists, whom he associated with a new philosophy of art-making, which he famously called "Arte Povera" (Poor Art, or perhaps better translated as Impoverished Art). Like Bohm, the text is recognisably of another era. Celant argued that the "true" artist, exemplified by Marcel Duchamp, is obliged to remain outside the system. If not, "the artist, the new apprentice jester, is … called upon to produce fine commercial merchandise, offering satisfaction to sophisticated palates." His articulation of Arte Povera includes the declaration:

> So on the one hand, we have an attitude to be defined as 'rich' since it is osmotically connected to the enormous instrumental and informational possibilities that the system offers; an attitude that imitates and mediates the real creates the dichotomy between art and life, public behavior and private life. And on the other hand, we have 'poor' research … This is a way of being that asks only for essential information, that refuses dialogue with both the social and the cultural systems, and that aspires to present itself as something sudden and unforeseen with respect to conventional expectations: an asystematic way of living in a world where the system is everything. Such an attitude … is intent upon retrieving the factual significance of the emerging meaning of human life. It's a question of an identification between man and nature, but with none of the theological purposes of the medieval *narrator-narratum*; the intention, quite to the contrary, is pragmatic, and the goal is liberation, rather than any addition of ideas or objects to the world as it presents itself today.

Celant's vision of art, shorn of the coat of 1960s polemic, is strikingly parallel to a conventional philosophical vision of science, which seeks identification between "man and nature", driven to understand the "factual" significance of our existence. Arte Povera, like Slow Food, was a radical rejection of the "system". If science is to revive, perhaps an equally radical stance is required.

I am struck by the notion that whereas (some) artists have wrestled with the importance of new ways of seeing, analogous discussions are (to my knowledge) nowhere to be found among scientists. Indeed, it is thanks to Robert Owen that I came across Celant's essay, recently reprinted to commemorate his death last year[2].

Whereas science and art will always have their differences, often communicated by different languages, both professions have something to learn from each other. Viewed from the scientific side of the linguistic border, art-speak is often obscure, with a propensity to purloin scientific concepts and reissue them in a half-garbled form, thereby gilding the work with an aura of the other. (Interestingly, the converse practice: art-speak in scientific publications, is hard to find, save

some celebrated cases; another story worth telling elsewhere). However, a deeper dive, into Celant, or Owen (or Cohen) reveals a humility and dedication to the primacy of deep, creative thought over the ”valuable”. It is time for scientists to abandon the widely-held view that art is secondary to science, forever hobbled by its fondness for imaginative thought over the empirical and its explicit admission of the irrational. It is time to rekindle the association between art and science which was openly acknowledged by scientists in earlier times. Bring on Slow Science and Scienza Povera. For even the most unfettered scientific poems may hit paydirt eventually. When asked by Gladstone what his new-fangled concept of electricity could offer society, Faraday famously answered

> Why sir, there is every possibility that you will soon be able to tax it.

His wit is undeniable, but has proven to be all-too prophetic in recent years. Despite his own well-documented integrity and humility, he crystallised a dangerous idea: science is money. Given the exorbitant prices exchanged on the international art market in recent years, so too is art. But that's not the point.

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Feature Articles

Creativity in the Art, Literature, Music, Science, and Inventions

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Abstract. This essay aims to stimulate reflection on the creativity characterising *Homo sapiens* in the different realms in which it occurs. Over recent decades scholarly research into creativity has extended the original concept, restricted to geniuses, to a broader field that encompasses the qualities and abilities of every individual, in line with a democratisation of the creative act. However, the aim of this contribution is to illustrate the creativity of geniuses, referring to examples in various fields, according to Poincaré's definition of connecting pre-existing elements into new combinations that are novel and useful. The objective of this study is to show that pre-existing elements can be found in works of art, literature, poetry, and music, as well as in scientific discoveries or inventions. Having demonstrated the existence of concrete and real analogies in the various – and apparently profoundly different – fields of human creativity, a second objective was to construct a convincing proof of a notion of a culture characterised by an essential unity, without any separation between humanities and sciences. I trust that the analysis of the creative acts that generated Picasso's *Les Demoiselles d'Avignon*, Michelangelo's *Vaticano Pietà*, Primo Levi's *The Periodic Table*, Giacomo Leopardi's *L'infinito* (*The infinite*) and Wisława Szymborska's *Liczba Pi* (*Pi*), the beginning of Beethoven's *Fifth Symphony* and Brahms' *Fourth Symphony* the finale of Stravinsky's *Sacre du Printemps*, the discovery of X-rays by Wilhelm Conrad Röntgen and of the therapeutic properties of lithium salts for psychiatric disorders by John Frederick Joseph Cade, the invention of incandescent light bulbs by Thomas Alva Edison and many other inventors and of the electronic television by Philo Taylor Farnsworth, may succeed in achieving the first objective and, by extension, the second also.

Keywords: creativity, genius, cultural unity, science, art, literature, music, inventions.

1. INTRODUCTION

It is well known that the literature on creativity has significantly evolved from the initial approach focused only on creativity as a peculiarity of geniuses in the various domains of human activity to what it has recognised as a democratisation of the phenomenon.[1-6] In particular, in a recent very accurate and in-depth study by Corazza, a detailed recognition of the literature about this passage from creativity by only geniuses to a broader meaning including qualities and abilities by every individual has been done.[7] In the same paper,

besides discussing this phenomenon of "creativity for all", some other very interesting topics are considered, as the mental processes associated with this broad vision of creativity and even the socio-cultural aspects.[8] Indeed, the state of the art of the literature allows individuating another further extension of the domain where creativity takes place, in the sense that creativity not only belongs to every human being, but it is also going to become the most important skill for the hyper-technological societies of the future.[9] Considering this enlarged vision of creativity, it is possible to extend the classical definition according to which creativity requires both *originality and effectiveness*, in order to contemplate several other requirements, as novelty, utility, aesthetics and authenticity.[10-13] In this context, according to the work by Weisberg who focused the attention on the *intentional novelty* as the unique criterion eliminating the *effectiveness* and trying to bypass the question of value assessment to individuate the *novelty* and/or *originality*, we may recognise three pragmatic definitions of creativity as reported in the literature.[7,14] According to this background, which refers to creativity in a static view, many scholars have investigated the topic of a dynamic concept of creativity, where the adjective *potential* is put before *originality and effectiveness*, with the aim of taking into account all the *inconclusive outcomes* and stressing the dynamic nature of creativity, as selecting a focus, generating the outcomes through complex processes where *inconclusive outcomes* play an important and decisive role, assessing them, and finally transforming the outcomes to knowledge.[7] Independently on this wide vision of creativity, it is worthwhile to recall that, despite the new achievements on the creativity concept above resumed, it remains valid one of the most interesting and comprehensive definitions of creativity which underline *originality and effectiveness*, like that described by Poincaré according to whom creativity can be summarised as the capacity of connecting pre-existing elements into new combinations that be useful.[15] This idea was at the centre of a very interesting talk given by Umberto Eco in Florence, Italy for the Nobel Foundation in 2004 which is titled *Combinatoria della creatività* (*Combinatory of creativity*).[16] It is interesting to notice that the "usefulness" to which Poincaré refers is more appropriately relative to the beauty rather than to the effectiveness. Of course, he thinks of beauty not in a strictly aesthetic sense, but rather in the view of mathematicians, that is something associated with elegance, harmony, the economy of signs, operational correspondence to the aims.[17] It is curious and intriguing that this idea of connecting things thought at the beginning of the 20th century by Poincaré is repeated almost identically by one of the fathers of the ICT industry, Steve Jobs, who defined creativity in a very concise and incisive way. "Creativity is just connecting things. When you ask creative people how they did something, they feel a little guilty because they didn't really do it, they just saw something. It seemed obvious to them after a while. That's because they were able to connect experiences they've had and synthesize new things. And the reason they were able to do that was that they've had more experiences, or they have thought more about their experiences than other people. Unfortunately, that's too rare a commodity. A lot of people in our industry haven't had very diverse experiences. So, they don't have enough dots to connect, and they end up with very linear solutions without a broad perspective on the problem. The broader one's understanding of the human experience, the better design we will have."[18] It is obvious that what is written until now about creativity strongly clashes with the etymology of the terms 'creativity' and 'to create', according to which the word comes from the Latin *creare* with shares with the word *crescere* (to grow in English) the root *kar*.[19,20] Indeed, the most common meaning of the word creativity is associated with the 'making from nothing', which is referred especially to God and that, in a figurative sense, becomes inventing, generating new and original things.[1-14] In the Sanskrit language *kar-tr* is 'the person who makes' (from nothing), the 'creator'.[19] Leaving this divine interpretation to philosophy and theology and reminding the previous introductory remarks on creativity, in particular as defined by Poincaré, it is worthwhile to mention that even Marcel Proust agrees, albeit more lyrically, with Poincaré. «The only true voyage of discovery, the only fountain of Eternal Youth, would be not to visit strange lands but to possess other eyes, to behold the universe through the eyes of another, of a hundred others, to behold the one hundred universes that each of them is; […]».[21] This lyric vision according to which the true discover, the fruit of the creative act, does not consist in finding new territories, but rather in seeing them with different eyes, perfectly matches with Poincaré's statement where the new territories are simply the pre-existing elements that the creator succeeds to connect generating *originality and effectiveness*, due to his ability in seeing them in a new combination thanks to his capacity in seeing them with different eyes. In conclusion, we can imagine creativity as an unlimited domain, because the pre-existing elements are in the number of billions and billions, and the combinatory calculus individuates several combinations truly tending to infinite. Therefore, we could define creativity as a bottomless pit; indeed, such pit has two negative aspects not coherent with creativity: it is dark, and one has to dive in a random fashion and therefore it is almost impossible

to sharpen one's different eyes, to use Proust's expression. Another metaphor for creativity is perhaps more appropriate: a sea that offers always new and unpredictable horizons. The present paper aims to illustrate with some examples taken from the art, music, literature, poetry, science, and the world of inventions how creativity continuously opens these new horizons simply by connecting pre-existing elements in some combinations that generate originality and novelty. By this approach we hope to succeed in extending the Poincaré's definition from the domain of the mathematical invention for which it was coined to all the other fields of the human creativity.[15] Indeed, we will revisit some fruits of creativity in the various domains of human activity we mentioned above discovering many analogies and to possibly conclude that there is no division, in contrast with Snow's idea, between science and art, science and literature, and that humanistic and scientific cultures are two complementary sides of the same medal hanging from the neck of *Homo sapiens*.[22-23]

2. CREATIVITY IN ART

To investigate the connection between pre-existing elements used to create new original and novel combinations in art, two examples will be illustrated, one from painting and the other from marble sculpture. The painting by Pablo Picasso *Les demoiselles d'Avignon* (https://www.moma.org/collection/works/79766) has been the subject of several studies focusing on its novelty and, above all, on the idea that it could represent the starting point of the nascent cubism.[24-25] The pre-existing elements can be recognised in the five women who had been already represented in the very famous painting cycle dedicated to *Les baigneuses* by Paul Cézanne. (See for example https://joyofmuseums.com/museums/united-kingdom-museums/london-museums/the-national-gallery-london/masterpieces-of-the-national-gallery/bathers-les-grandes-baigneuses-by-paul-cezanne/). It is still controversial whether this reference was in Picasso's mind or not at the beginning of his project to paint his *Demoiselles d'Avignon*.[26-27] Nevertheless, apart from the shapes of the bodies, clearly inspired by the nascent cubism, it is evocative the idea that the blue of the triangles, trapezoids, other irregular figures and some outlines of the bodies can be traced back to the blue of the water close to the bathers. In this view, the bathing women and the blue of the water are the pre-existing elements, and the new, unexpected, and amazing connection is the geometry of the nascent cubism. Among all the possible connections of these pre-existing elements – bathers and water – whether he is referring or not to Cézanne, Picasso sees with different and new eyes the shape of the bathing bodies and the blue-coloured geometries representing the water.

Another interesting case of connecting pre-existing elements into new combinations that generate beauty in art is the *Pietà* by Michelangiolo housed in *Saint Peter's Basilica,* Vatican City. (https://www.flickr.com/photos/jorge-11/48126571846) Here the pre-existing elements are the well-known sculptural groups called *Pietà* (*Compassion*) mainly made of wood and typical of Northern-Europe with the German term *Vesperbild*.[28] The tradition until Michelangiolo, both in painting and sculpture, was characterised by a rigid and geometric structure of the Madonna and Christ pair. The seated Madonna was the vertical element to whom the dead Christ in a horizontal position was opposed with a clear association with the geometry of the cross. All the paintings and sculptures focused on this iconography were characterised by rigidity: a striking example is the oil painting Pietà by Pietro Perugino housed at the Galleria degli Uffizi in Florence (https://www.flickr.com/photos/nikonpaul/31992383947) and executed a few years before Michelangiolo's *Pietà* in the Vatican.[29] Moreover, another example of the strong rigidity of the sculptures is in a *Vesperbild* by an anonymous German sculptor (ca. 1380-1400) conserved at *Liebieghaus Skulpturensammlung*, Frankfurt-am-Mein.[28] (https://www.foglidarte.it/luoghi-mostre-eventi/699-vesperbild-alle-origini-delle-pieta-di-michelangelo.html) Michelangiolo has in front of him the following pre-existing elements: the dead body of Christ which of course must remain in an almost horizontal position, the seated Madonna who has to hold her son. His extraordinary creativity is to combine these two figures to compose the group with two amazing novelties: softness replacing the rigidity and the horizontal/vertical contrast that tends to dissolve. The artist concentrates his action to give softness to the marble – it may seem like a paradox or oxymoron while it is an inspired new combination of pre-existing elements! – working briskly at the Madonna's drapery. Another virtuoso combination concerns the two arms of the Madonna, obvious pre-existing elements. The left arm is completely autonomous and does not even touch Christ and the left hand is directed towards us, as if inviting us to meditate with the typical gesture of an almost open hand exposing the palm.[30] In sculpting the right arm Michelangiolo succeeded in capturing an amazing combination by achieving both the softness and the realistic effect of supporting the heaviness of a lifeless body. He concentrates on the region of the marble that connects Mary's arm, the Christ's right arm and ribs: these

three elements had to be connected in an original, never seen before way. The creative genius of Michelangiolo is expressed with a combination in that area of the statue which produces a kind of an elongated "sausage" of flesh between the arm and the ribs due to the firm hold of the Madonna opposed to the weight of Christ's listless body.

3. CREATIVITY IN LITERATURE AND POETRY

Probably the most impressive example of the connection of pre-existing elements ingeniously combined to generate the creation of a literary work is *Il sistema periodico* (*The periodic table*) by P. Levi.[31] In this wonderful book the writer, a chemist, succeeds in creating one of the most original connections of pre-existing elements. Indeed, he starts from the chemical elementary elements of the Mendeleev's table, the bricks of the whole universe, and he associates to each of the selected elements – twenty-one in total – an autobiographical life experience. Two extraordinary combinations are worthwhile mentioning in relation to the aim of the present contribution. The first deals with story that we find in the central pages of the book – the eleventh, entitled *Cerium* – where the pre-existing chemical element, apparently completely removed from the dramatic experience of the lager deportation, becomes the fulcrum around which the theme of the "*saved and drowned*" is developed, building in a few pages one of the crudest and most realistic testimonies of the Shoah.[32] In the second – the book's last one, entitled *Carbon* – the masterful combination of pre-existing elements is that between carbon, the very atom of life and the author's act of writing: the continuous *panta rei* of the matter finds concrete realisation through a "labelled" carbon atom that passes from a compound to another travelling in space and time until it arrives in Levi's brain, driving his pen or typewriter to write the final full stop of both the story and the book. Keeping the focus on Levi's work, there is another excellent example of an almost jarring combination between two elements: a make-up item such as lipstick and its main chemical component called alloxan which is also found in chicken droppings, a kind of revival of the old Latin motto *aurum de stercore*! «The fact that alloxan, destined to embellish ladies' lips, would come from the excrement of chickens or pythons was a thought which didn't trouble me for a moment. […] I will go further: far from scandalizing me, the idea of obtaining a cosmetic from excrement, that is, *aurum de stercore* ("gold from dung"), amused me and warmed my heart like a return to the origins, when alchemists extracted phosphorous from urine.» (See ref. 31 English transl. story "*Nitrogen*", pp. 180-181). Some years before the publication of the book by the chemist-writer Levi, the Italian songwriter-poet Fabrizio De André combined the same elements in his very famous song *Via del Campo*: «*dai diamanti non nasce niente, dal letame nascono i fiori*» (transl. « *diamonds bring nothing, manure brings flowers* »).[33]

Concerning creativity in poetry, it is particularly interesting to analyse two different examples: the poems *L'infinito* (*The Infinite*) by the Italian G. Leopardi and *Liczba PI* (*Pi*) by the Polish W. Szymborska.[34-35] The theme of the infinite is a subject that drew the interest of philosophers, theologists, mathematicians, physicists and astronomers alike since the beginning of human civilisation. Indeed, even writers and poets have been fascinated by this topic, but the amazing ways that Leopardi finds to describe the infinite in his poem are truly unparalleled. Leopardi, according to Poincaré's definition of creativity, establishes several new combinations (see bold below) by connecting pre-existing elements.[15,35] The hedge as the limit of finiteness and the farthest horizon as a symbol of infinity; the visual boundless spaces opposed to the auditory, superhuman silences; the same silence and the voice both combined with the wind that moves the plants; the present time perceived through its sound, that is, the living season as opposed to eternity, dead, silent; the final paradoxical and oxymoronic connection between the drowning of the thought on the one hand and the shipwreck of the thinker at the other. How many combinations of these pre-existing elements could the poet choose? We can very well say, an infinite number! But Leopardi selected the ones marked in bold below in the poem quoted in its entirety (Box 1).

We could venture that the reason why among the various possibilities for such combinations only some – very few – extraordinary ones, the fruit of the ingenious creativity, succeeded in generating eternal cultural products, resides in a kind of evolutionary theory involving natural selection. The literary "species", the fruit of creativity, that show the best "fitness" to the judgment of the posterity survive and do not become extinct, all the others do not survive the natural selection process and end up in oblivion. Something similar has been established by a group of scientists who have recently concluded that mutations and gene variability explain why a lot of music of a single genre with various species is produced in each period. Then, the free choices of the listeners determine the pressure of the external environment – fitness – and cause some species to become extinct, making certain others durable and transmissible for centuries.[37]

Another fantastic example of an ingenious and incredible combination of pre-existing elements used to

Box 1.

L'infinito	The infinite
Sempre caro mi fu quest'ermo colle, e **questa siepe**, che da tanta parte **dell'ultimo orizzonte** il guardo esclude. Ma sedendo e mirando**, interminati** **spazi** di là da quella, e **sovrumani** **silenzi**, e profondissima quiete io nel pensier mi fingo; ove per poco il cor non si spaura. E **come il vento** **odo stormir tra queste piante**, io quello **infinito silenzio a questa voce** vo comparando: e mi sovvien l'eterno, e le morte stagioni, **e la presente** **e viva, e il suon di lei**. Così tra questa immensità **s'annega il pensier mio:** **e il naufragar m'è dolce in questo mare**.	Always dear to me was this solitary hill and **this hedge**, that excludes so great a part **of the farthest horizon** from my sight. But sitting and gazing, **boundless** **spaces** beyond it, and **superhuman** **silences**, and deepest quiet, I envision in my mind; where almost awed is the heart. And as the wind **I hear sighing through these plants**, I that **infinite silence to this voice** go comparing: and I remember eternity, and the dead seasons, and **the present** **and live one, and its sound**. So in this immensity **my thought is drowned:** **and sweet to me is shipwreck in this sea**.

build a wonderful, unique, and original poem is *Liczba Pi* (*Pi*) by the Polish poetess W. Szymborska.[34] In this poem there are a number of brilliant connections of many pre-existing elements to constitute an extraordinary combination: a transcendental number, the number Pi, is the pretext to lyrically suggest reflections on mankind, nature, life, consciousness, eternity. A poetic way to look at an ancient philosophical subject, that of finiteness and infinity, thanks to the very ingenious combinations: "*five nine two* because it never ends", "the longest snake on earth calls it quits at about forty feet", but it "doesn't stop at the page's edge" and finally it nudges the "sluggish eternity to continue" (Box 2). Starting from an ordered, infinite set of integers that seem randomly put one behind the other, and which instead represent a very tangible concept, i.e., the ratio between the length of a circle and its diameter, the poetess combines some elements to evoke feelings and emotions perfectly succeeding in making warm and throbbing what is considered by everybody one of the coldest and driest objects, a number! Another perfect demonstration of what Poincaré intended for connection of pre-existing elements to generate an ingenious combination.

4. CREATIVITY IN MUSIC

Trying to apply Poincaré's definition of creativity to music is a truly difficult challenge, since the pre-existing elements are the most abstract and intangible. So far, the pre-existing elements have been well tangible and concrete, like marble or coloured pigments, or intangible like words, but still evoking specific concepts or things. In music the combination is made of sounds whose physical frequency and mathematical duration give melody and rhythm, whereas Fourier's spectral analysis and simultaneous presence of different frequencies give timbers and harmony, respectively.[37] It is almost impossible to show how some combinations of sounds originate music that becomes original and encounters the appreciation of the listeners: therefore, the strangest and somehow most absurd combination of pre-existing elements which will be illustrated as capable of originating novelty and originality is that of sounds and silence, that is, the use of breaks in music generating something truly original and ingenious. Every musical piece obviously starts from silence, but we cannot conclude that the combination between the silence before the first sounds of the musical piece represents a demonstration of an ingenious combination of pre-existing elements, i.e., silence and sound. Nevertheless, there are some cases where the composer intentionally combines a break with the first sounds to start his or her musical piece in a very original way, as if the music gushed out of nowhere. Two very famous examples where Poincaré's definition could be applied to creativity in music are the beginning of Beethoven's 5th Symphony and that of Brahms's 4th Symphony. How did Beethoven succeed in starting his Symphony so that just based on its beginning it was celebrated as "the destiny knocking on the door"? By a combination of a quaver rest, followed by three identical quaver notes and by a final minim note with a crown. The knocking on the door by the destiny is a rest, that is, a silence! The combination between the rest and the four

Box 2.

Liczba Pi	Pi
Podziwu godna liczba Pi *trzy koma jeden cztery jeden*. Wszystkie jej dalsze cyfry też są początkowe *pięć dziewięć dwa*, ponieważ nigdy się nie kończy. Nie pozwala się objąć *sześć pięć trzy pięć* spojrzeniem, *osiem dziewięć* obliczeniem, *siedem dziewięć* wyobraźnią, a nawet *trzy dwa trzy osiem* żartem, czyli porównaniem *cztery sześć* do czegokolwiek *dwa sześć cztery trzy* na świecie. Najdłuższy ziemski wąż po kilkunastu metrach się urywa. Podobnie, choć trochę później, czynią węże bajeczne.	The admirable number Pi: *three point one four one*. All the following digits are also initial, *five nine two* because it never ends. It can't be comprehended *six five three five* at a glance, *eight nine* by calculation, *seven nine* or imagination, not even *three two three eight* by wit, that is, by comparison *four six* to anything else *two six four three* in the world. The longest snake on earth calls it quits at about forty feet. Likewise, snakes of myth and legend, though they may hold out a bit longer.
Korowód cyfr składających się na liczbę Pi nie zatrzymuje się na brzegu kartki, potrafi ciągnąć się po stole, przez powietrze, przez mur, liść, gniazdo ptasie, chmury, prosto w niebo, przez całą nieba wzdętość i bezdenność. O, jak krótki, wprost mysi, jest warkocz komety!	The pageant of digits comprising the number Pi doesn't stop at the page's edge. It goes on across the table, through the air, over a wall, a leaf, a bird's nest, clouds, straight into the sky, through all the bottomless, bloated heavens. Oh how brief — a mouse tail, a pigtail — is the tail of a comet!
Jak wątły promień gwiazdy, że zakrzywia się w lada przestrzeni!	How feeble the star's ray, bent by bumping up against space!
A tu *dwa trzy piętnaście trzysta dziewiętnaście* *mój numer telefonu twój numer koszuli* *rok tysiąc dziewięćset siedemdziesiąty trzeci szóste piętro* *ilość mieszkańców sześćdziesiąt pięć groszy* *obwód w biodrach dwa palce* szarada i szyfr, w którym *słowiczku mój a leć, a piej*	While here we have *two three fifteen three hundred nineteen* *my phone number your shirt size* *the year nineteen hundred and seventy-three the sixth floor* *the number of inhabitants sixty-five cents* *hip measurement two fingers* a charade, a code, in which we find *hail to thee, blithe spirit, bird thou never wert*
oraz *uprasza się zachować spokój*, a także *ziemia i niebo przeminą*, ale nie liczba Pi, co to to nie, ona wciąż swoje niezłe jeszcze *pięć*, nie byle jakie *osiem*, nie ostatnie *siedem*, przynaglając, ach przynaglając gnuśną wieczność do trwania.	alongside *ladies and gentlemen, no cause for alarm*, as well as *heaven and earth shall pass away*, but not the number Pi, oh no, nothing doing, it keeps right on with its rather remarkable *five*, its uncommonly fine *eigh*t, its far from final *seven*, nudging, always nudging a sluggish eternity to continue.

subsequent notes generated what is considered one of the most powerful beginnings of a musical work.[38]

The music gushes out of nowhere and it makes it in an abrupt and violent way thanks to the combination of the pre-existing elements above described. But the music can also gush out of nowhere like a silence that softly and gently becomes music, as if silence contained somehow music. Brahms succeeds in making this miracle with the beginning of his 4th Symphony: Beethoven started with the silence – the quaver rest – here Brahms starts with a quasi-silence, with all the instruments having a crotchet rest while first and second violins playing an upbeat B crotchet with anacrustic rhythm. The creativity manifests itself with this ingenious combination and the conductor's ability consists of realising this gushing out of music from nothing: one of the best realisations is by Carlos Kleiber, who brings music out of thin air with a masterful gesture.[39] Very appropriately, they have written that "this opening is a prime example of Brahms's natural ability to compose within the strictest and oldest of structures, yet with a fluid, modern sound that belies its rigidity".[40-42] One could conclude that in this case the quasi-nothing represented by those rests and by that short upbeat note he creates something truly original, novel, effective, and useful.[10-15] But the rests, the silence, can also be an ingenious inven-

tion for a sensational final twist: the example that comes to mind is represented by those three rests – dotted quaver, crotchet, dotted quaver – after the rapid ten-note sequence by flutes that precedes the thunderous finale chords of the *Sacre du printemps* by Igor' Fëdorovič Stravinskij.

5. CREATIVITY IN SCIENCE

Two examples of a combination of pre-existing elements to create new progress in science by means of an important and fundamental discovery will be illustrated. It is interesting to notice that a special role was played also by serendipity, i.e., "the faculty or phenomenon of finding valuable or agreeable things not sought for".[43] Therefore, the pre-existing elements can be found with some serendipity, but creativity consists of connecting and combining them so as to lead to a new discovery. In relation to this aspect, it is worth mentioning that in science, unlike in the other branches of the human activity described in this work, the above-mentioned combination/connection is associated with the importance of inductive reasoning in 'creating' creativity. We could state that inductive reasoning is some kind of prerequisite for the selection of the pre-existing elements to be combined; in other words, inductive reasoning is the sieve that separates wheat from chaff, but the 'creative jump' is linked to the ability of *Homo sapiens* to combine the wheat grains to give an 'original, novel, and useful bread'!

The first example is the discovery of X-rays by Wilhelm Conrad Röntgen in 1895. It is well known that several pre-Röntgen experiments had evidenced radiations of some kind, but as well characterised as to differ from cathode rays and fluorescence radiation.[44] Firstly, there were Morgan's experiments in 1785, followed by many other observations involving such great scientists as H. Davy, M. Faraday, P. Lenard, W. Crookes, F. Sanford, H. Helmholtz, H. Hertz, I. Puluj, N. Tesla.[45-47] It is difficult to ascertain the true origin of Röntgen's X-rays discovery, but the received hypothesis, as reported by the most important biographers, is that he succeeded in isolating the effects of fluorescent radiation visible to the naked eye, by wrapping in black cardboard a Crookes tube where experiments of electric discharges on very rarefied gases were carried out. Apparently, the differentiation with the already known cathode rays occurred by serendipity – as is testified by Lenard in 1888 who in his experiments observed the effects of cathode rays (perhaps, unknowingly, even of X-rays!) on photographic plates outside the tube in the region of the cathode and measured their penetration through various materials.[47-49] Indeed, at 1 m distance from the cathode of the Crookes tube there was a fluorescent screen painted with barium platinocyanide that showed a green glow even when nothing was apparently coming out of the tube. Röntgen thought to connect and combine the following two pre-existing elements: (i) no visible fluorescence coming out from the tube due to the black cardboard, (ii) a fluorescent screen painted with barium platinocyanide placed at a considerable distance far from the tube compared to Lenard's experiments. From this connection, Röntgen drew a novel idea, the fruit of his creativity: some invisible rays coming from the tube were passing through the cardboard and succeeding in crossing 1 m of air to react with the barium platinocyanide making it fluoresce. Two months of further experiments went by when he thoroughly investigated these new invisible rays before publishing his first paper. He decided to name the rays "X" same as the mathematical unknown.[50] But the combination of pre-existing elements did not stop here: during the experiments, Röntgen realised that one of the most impressive characteristics of such mysterious rays was the ability to go through some materials, such as paper, books, wood, but not others such as metals and stone. Connecting this experimental evidence, he designed three ground-breaking experiments: he made the invisible rays going through three different objects and placed a few inches beyond each object a photographic plate and then darkened the whole room. The first object was a wooden box that contained the small metal weights of a balance; the second consisted in the various parts of his shotgun's barrel; and the third was the hand of his wife. The amazing combination of pre-existing elements originated in a very short time three extraordinary applications of the newly-discovered X-rays: metal detector, quality control in the metal industry, and radiology and radio-diagnostics in medicine!

The second example deals with the discovery of lithium-ion therapy for manic depressive patients by the Australian psychiatrist Dr John Frederick Joseph Cade who worked at the Bundoora Repatriation Mental Hospital in Melbourne. Here the connection and combination of pre-existing elements are truly unbelievable, also because the means and laboratories available to Cade at that time were verging on what has been named "ramshackle pantry".[51] The first element from which Cade started his research was a conviction, the classical hypothesis to be validated by experiments: depressive diseases (i.e., manic syndromes and bipolar disorders) could be due to chemical metabolic disorders that should determine a change in the chemical composition of the urine of his patients compared to that

of healthy people. With the aim of checking the validity of such assumption, he injected urine of both ill and healthy people into the abdominal cavities of Guinea pigs and, probably due to some preliminary not statistically reliable results, he found that urine from patients with depressive diseases was more toxic to the Guinea pigs than that of healthy people.[51-53] This unreliability of the experimental result was the serendipity touch, since it convinced Cade about the rightness of his hypothesis and led him to focus on the two main nitrogenous components of urine, for which it was important to ascertain the possible specific lethal constituent that, coherent to the hypothesis and the preliminary unreliable experiments, could be particularly concentrated in the urine of ill patients.[55] The treatment with the urea solutions led to the same effect of urine from ill patients, but it was impossible to explain the greater toxicity of the urine of manic patients simply in terms of higher concentrations of urea. Indeed, urea is toxic for Guinea pigs from a certain threshold of concentration and there was no difference in the effect of the urine from healthy or ill people since it was the unreliability of the experiment described, a true mistake, that was going to generate the discovery! Cade had the idea to add the second constituent, uric acid, to test whether it had a synergistic – positive or negative – effect, or not.[54,55] In his first article in 1947, Cade noted that uric acid had a slightly enhancing effect on the toxicity of urea.[56] This result prompted him to continue this research strategy, but unfortunately, it was impossible to increase the concentration of uric acid due to its very scarce solubility in water (0.06 g/L equivalent to $3.57 \cdot 10^{-4}$ M compared to 1,193 g/L equivalent to ca. 20 M of urea). Therefore, Cade selected lithium salt as it was the most soluble salt of uric acid, to increase the concentration of the possible enhancer of urea toxicity. To Cade's surprise, when he injected the Guinea pigs with lithium urate in conjunction with urea, the toxicity was reduced rather than enhanced, suggesting that the lithium could have been protective. Cade further explored this lead by injecting the Guinea pigs with lithium carbonate in conjunction with urea, and once more observed a reduced toxicity. He concluded that lithium itself provided a protective effect against the action of urea. This belief then prompted him to wonder whether lithium per se would influence his Guinea pigs. Injecting them with large doses of lithium carbonate, he found them to become lethargic and unresponsive. Now we can deduce that the lithium ion itself had a protective function against the convulsant death caused by toxic doses of urea.[55,56] A mistake and a touch of serendipity coupled with the combination and connection of some pre-existing elements by the creative mind of Dr Cade: the toxic effect of urea and uric acid, the low solubility of uric acid, the higher solubility of lithium urate, the even higher solubility of Li_2CO_3 ($1.75 \cdot 10^{-2}$ M) resulted in a novel, original, useful, and effective discovery that had to wait another twenty years to become a worldwide-accepted therapy. But this is another story.

6. CREATIVITY AND INVENTIONS

Even the inventors can be considered creative in the sense illustrated in the previous section: two examples will be described to demonstrate once again the creative act always as a combination of pre-existing elements. The first case concerns the invention of the incandescent light bulb; despite the fact that the invention is attributed to Thomas Alva Edison the story is much more complex and involves several more inventors who worked for many years between the end of the 19th and the beginning of the 20th centuries.[57] These inventors combined pre-existing elements into an ingenious invention that led the American writer Oliver Sacks to entitle "Light for the masses" one of the chapters of his extraordinary book *Uncle Tungsten: Memories of a Chemical Boyhood* and here I will illustrate the pre-existing elements and the ingenious combination and connection thereof. The pre-existing elements were the following: (i) Joule effect condensed in the formula $Q = I^2 \cdot R \cdot t$, (ii) black body radiation, (iii) heat transfer by conduction, convection, and radiation, (iv) oxidation reactions, and (v) matter phase transitions. The combination of the first two elements led many inventors to design an ingenious new object consisting of a filament of matter that, when heated by an electric current at high temperature, emitted radiation in the visible region of the spectrum as a black body. The third element combined with the fourth convinced these ingenious men to put such filaments in a vacuum to enhance radiation against conduction and convection and inhibit oxidation processes that caused the light bulbs to have a very short lifetime. The combination with the fifth element was the most ingenious and came later: instead of the vacuum an inert gas like Argon was used. This prevented oxidation and simultaneously favoured conduction and convection, losing a little bit of radiation power, but lowering considerably the sublimation of the tungsten filament, because of the decrease in heat produced during this transition phase. As a matter of fact, sublimation reduced the life of the filament and caused a blackening of the glass due to condensation of tungsten vapour: in this way, for the first time in the history, humans succeeded in illuminating the dark not by

chemical combustion, but by radiation-induced electricity. It was the beginning of a new era.[59]

The second example is the invention of the electronics-based television. Probably this is the best example to illustrate Poincaré's definition of creativity. Philo T. Farnsworth was an extraordinary inventor and the idea to design a way to capture images in movement and reproduce them on a fluorescent screen came to his mind after observing a farmer ploughing a field.[60] The pre-existing elements were the following: (i) certain materials, submitted to an electrical potential when illuminated have the properties to generate an electrical current proportional to the illumination intensity, due to a decreased resistance induced by the light, (ii) the electrical signals so obtained can be transmitted at distance by means of radiofrequency waves, (iii) the cathode rays produced in a Crookes tube manage to illuminate the phosphors in the internal side of a fluorescent screen, and (iv) the electrical signals of element (ii), that are a faithful mirror of the image collected, can be used to attenuate the cathode rays according to the inverse proportionality law (i.e., strong electrical signal = high illumination of the photosensitive material = weak attenuation of the cathode ray = high illumination of the television screen). The extraordinary connection of these four elements was invented ingeniously by Farnsworth thinking of a fast-moving plough. According to the Italian standard, the plough (cathode tube emitting cathode rays) ploughed the field (scanned the fluorescent screen) with 625 furrows (lines) repeated 50 times per second! The lines were constituted of alternated microscopic black (no light) and white (light) spots moving exactly in the same way they were collected by the camera. The viewer does not notice this process of image composition due to the double phenomenon of the high speed of the electronic brush and the persistence of the image on the retina of the human eye ($1/16^{th}$ of a second).

7. CONCLUSIONS

Starting from the recent review of the literature on creativity research and recollecting the ever current definition by Poincaré for the creativity in the mathematical invention, the present contribution tried to demonstrate that the creativity jumps typical of geniuses in the various domains of the human activity can be aptly described in terms of combination and connection of pre-existing elements to generate novelty, originality, and effectiveness.[1-15] In particular, some examples of creativity outcomes in art, literature, poetry, music, science, and the world of the inventions were reviewed focusing on the pre-existing elements and on the ingenious combination and/or connection between them to generate the creative jump. The examples illustrated here, while belonging to apparently separate fields of human creativity, reveal, on the contrary, a common matrix precisely in the creative act by the genius who conceived it. In the end, we discovered the common traits of the creation of Michelangiolo's *Vaticano Pietà*, Beethoven's Fifth Symphony's beginning, Primo Levi, Leopardi or Szymborska's works, the X-rays' discovery, or television and light bulbs inventions. Besides confirming the shrewd intuition inherent in Poincaré's definition of creativity, it was demonstrated that the analogies found in the genesis of the above-mentioned products of creativity were very strong, enabling us to conclude that there is no division, in contrast with Snow's idea, between science and art, science and literature, and that the humanities and sciences are two complementary sides of the same medal hanging from the neck of the *Homo sapiens*.[22-23]

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Research Articles

Singlet Dioxygen $^1O_2$, its Generation, Physico-chemical Properties and its Possible Hormetic Behavior in Cancer Therapy

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Abstract. Singlet dioxygen $^1O_2$ is one excited state among the three other possible spectroscopic states of molecular oxygen. Here, we first describe the use of published spectroscopic data and thermodynamic modeling based on irreversible entropy production. Such concepts are further applied to the synthesis of singlet dioxygen and its reactions with crucial biological molecules. In a last section, we suggest that singlet dioxygen and ozone may be responsible for the success of radiation therapy, that has been used to treat cancer successfully for over 120 years. Its precise mechanism of action remains controversial. We thus aim to clarify the role of singlet oxygen in radiotherapy and chemotherapy. A partial conversion of ionizing radiation in the body into thermal photons could be assumed. The antitumor effect may involve these thermal photons, such as the one delivered by red/infrared sources. Thermal photons (wavelengths of 635 nm and 1270 nm) convert triplet dioxygen into singlet dioxygen by changing the spin of its outer electrons. Despite its short half-life, Singlet dioxygen is responsible for the activation of multiple free radicals (such as hydrogen peroxide), which may target proteins and DNA, induce either apoptosis or oxidative phosphorylation. At moderate concentrations, thermodynamic data suggests that singlet dioxygen may readily react with water to form a potent pro-apoptotic molecule (ozone), thus decreasing cancer growth. However, at high concentration cytotoxic effects against all kind of cells occurs. This strongly suggests a non-linear hormetic behavior of singlet dioxygen. It is also proposed that cytotoxic chemotherapy induces the same free radicals that singlet dioxygen does. There are also other ways to enhance the production of singlet dioxygen, such as phototherapy using Methylene Blue for instance. As a source of reactive oxygen species (ROS), singlet oxygen could thus be a common agent active both in radiotherapy and chemotherapy. It is probable that the activity of radiation therapy and chemotherapy may be mediated by the conversion of triplet to singlet oxygen. This may explain the oxygen effect such as described in radiotherapy and chemotherapy.

Keywords: cancer, phototherapy, Warburg's effect, radiation therapy, singlet oxygen, chemotherapy, oxygen effect.

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INTRODUCTION

Less than two months after the discovery of X-rays by Wilhelm Röntgen in 1895, Leopold Freund treated, successfully, a child with a large nevus, a benign skin lesion[1]. In the following months, there were multiple reports of the efficacy of radiation therapy in the treatment of both benign and malignant lesions. Radiation therapy (RT) is a therapy using ionizing radiation to control or kill inflammatory and cancer cells. RT has been extensively used for the treatment of inflammation, but this indication is slowly disappearing because of the risk of radiation-induced malignancies[2]. RT may be curative in several types of cancer if they are localized to one limited area of the body. Several shaped radiation beams coming from several angles of exposure intersect at the tumor to spare normal tissues (such as skin or organs that radiation must pass through to treat the tumor). This provides a much higher absorbed dose there than in the surrounding healthy tissue. RT kills normal cells, and every radiation oncologist knows the dose not to trespass to the normal tissues.

Ionizing radiation has been published to target the DNA leading to cell death. In the laboratory setting, the damage caused by ionizing radiation to the DNA is immediate and consists of single or double-strand breaks and mutations[3]. A correlation exists between the toxicity of RT to the normal cells and the damage to the DNA[4].

Unlike the laboratory setting, there is no immediate sign of death for cancer cells in clinical practice. In the minutes following a cardiac infarct, there is an increased level of cardiac enzymes, such as troponin, in the blood plasma[5]. Assessment of the treatment response after RT occurs, not minutes or even days but weeks after the inception of treatment[6].

Recently, Radman demonstrated that the prime target of radiation is not the DNA as previously thought but the proteasome. The cell dies because of oxidative damage to its proteins[7]. The polymerases may repair the concomitant damage to the DNA.

This paper aims to suggest existence of a link between RT and production of singlet dioxygen mediated by thermal photons. Radiation may also affect the activity of water around proteins or DNA and change mitochondria activity. Herein, we will not try reviewing the past 50 years in mechanistic, spectroscopic, computational, and biological studies of singlet oxygen. This topic is covered in great details in a recent textbook[8]. Our interest is rather to work in an historical perspective with focus on rather old concepts that will be revisited through the lens of the entropy concept[9-11]. Moreover, we are perfectly aware that singlet dioxygen reacts by two distinctive pathways (Type I and Type II mechanisms), and causes damage to biomolecules, materials such as polymers, food, paints etc. Amino acids, nucleic acids, unsaturated molecules (e.g.; membranes) also react with singlet oxygen to yield decomposed products and consequently to cause cell death. But, all these important properties, which rationalize the toxic and fatal behavior of $^1O_2$ to organisms completely neglects the fact that many biological processes, display a biphasic or triphasic response to exposure to increasing amounts of a substance or condition such as radiation. Even if this hormesis model of dose response is still vigorously debated[12], it seems worth investigating if it could apply to the biological response of singlet dioxygen. Moreover, in the spirit of putting more physics in biological or medical thinking, part of the article will be devoted to a reminder of the electronic structure and spectroscopic properties of these species deriving from molecular oxygen. Finally, we will focus mainly on healing cancer, even if the ideas exposed here could be extended to other diseases.

THERMAL PHOTONS ARE EFFECTIVE AGAINST CANCER AND INFLAMMATION

The metabolism of the cancer cells has been extensively studied since the seminal work of the German scientist Otto Warburg[13]. Warburg's effect is, in fact, the cause of every hallmark of cancer, such as the proliferation of cells, angiogenesis, or immortality[14]. Cancer is not the only disease involving Warburg's effect, as this effect is also crucial in inflammation[15] or Alzheimer's disease[16]. Alleviating Warburg's effect decreases cell proliferation[14].

Non-ionizing radiation has been developed successfully in the treatment of both benign and malignant tumors. Delivery of hyperthermia is possible using non-ionizing radiation such as ultrasound, microwave, or most commonly infrared (thermal) photons. Red and infra-red photons have also been used in the treatment of inflammation.

It is a well-accepted fact that a practice does not need total mechanism clarity to operate. More than 6500 publications registered on PubMed from LLLT keyword (*Low-Level Light Therapies*) covering cancer[17] wound healing[18], inflammation and pain management[19], muscles and joints injuries[20] as well as nerve regeneration[21], traumatic brain injuries[22], depression and anxiety[23] and more recently neurodegenerative such as Alzheimer and Parkinson diseases[24] as well as Age-Related Macular Degeneration[25].

THE THREE FORMS OF MOLECULAR DIOXYGEN

Dioxygen is a very peculiar molecule whose chemical behavior cannot be explained using conventional octet's rule[26]. Such rule generally applies to any molecule built from atoms belonging to the second period of the periodic table of the elements. Let N be the total number of atoms, E, the total number of valence electrons, Q the number of atoms other than hydrogen, and C, the number of cycles. It then mathematically follows that the number of single bonds should be S = N + C – 1, the number of lone pairs should be L = E – 3×Q – N, and the number of multiple bonds should be M = 3×Q – E/2 – C + 1 – E%2[27]. Here E%2 = 0 or 1 if E is respectively even or odd. For dioxygen O_2 characterized by E = 6 + 6 = 12, N = Q = 1 + 1 = 2, C = 0, the rule predicts that S = 2 + 0 – 1 = 1 (one single bond), L = 12 – 3×2 – 2 = 4 (four lone pairs) and M = 3×2 – 12/2 – 0 + 1 = 1 (one double bond). This corresponds to the classical notation :O:=:O: found in every elementary chemistry textbook. The trouble is that such a formula is utterly wrong as it predicts that dioxygen, having an even number of electrons, should be a diamagnetic molecule in its ground state. Experiments, on the other hand, show that dioxygen is rather a paramagnetic molecule in its ground state, diamagnetic states corresponding to excited states.

Such a deep mystery could be resolved by writing Lewis's structures after the removal of two electrons ($O_2^{2\oplus}$ ion with E = 10) or the addition of two electrons ($O_2^{2\ominus}$ ion with E = 14)[28]. For the dication, Langmuir's rules predicts that the number of single bonds does not change (S = 1), but that L = 10 – 3×2 – 2 = 2 (two lone pairs) and M = 3×2 – 10/2 – 0 + 1 = 2 (one triple bond). This corresponds to the classical notation, $^{\oplus}$:O≡O:$^{\oplus}$, meaning that the two electrons in the highest occupied energy level are of anti-bonding character, as removing them leads to the apparition of an additional chemical bond. Concerning, the dianion, the same rules predicts that L = 14 – 3×2 – 2 = 6 (six lone pairs) and M = 3×2 – 14/2 – 0 + 1 = 0 (no multiple bond, i.e. $^{\ominus}$:Ö:–:Ö:$^{\ominus}$). This means that the lowest unoccupied energy level is also of anti-bonding character, as adding 2 electrons there leads to the transformation of the double bond into two lone-pairs and a single bond. So, using just the well-established octet's rule, it could be anticipated that the states of the highest energy (occupied and unoccupied) in dioxygen are of similar nature (anti-bonding character). This strongly suggests that these two states have the same energy (degeneracy) with a single unpaired electron in each state, •:O: —:O:•. This explains the observed paramagnetism of dioxygen in its ground state.

Further development of quantum mechanics and group theory has confirmed the validity of such a picture. Accordingly, owing to its high symmetry ($D_{\infty h}$ cylindrical symmetry), molecular orbital (MO) theory predicts that dioxygen has a doubly degenerated HOMO (highest occupied molecular orbital) or LUMO (lowest unoccupied molecular orbital). In other words, writing structures obeying the octet's rule is an easy graphical way to get good solutions for Schrödinger's equation.

From MO-theory, we also learn that, owing to the phenomenon of resonance, obeying octet's rule can be of a dynamic nature. Thus, starting from the static solution (S = 1, L = 4, M = 2), we get the dynamic solution (S = 1, L = 5, M = 1), after transformation of the double bond into a delocalized lone pair:

:Ö:$^{\ominus}$—:O:$^{\oplus}$ ↔ $^{\oplus}$:O: —:Ö:$^{\ominus}$

Here, violation of the octet's rule occurs at a given time. However, after averaging in time, there is no possibility of distinguishing between the two oxygen atoms owing to the $D_{\infty h}$ cylindrical symmetry. This restores octet's rule, but in a dynamic sense.

Consequently, for a good understanding of dioxygen chemistry, it appears necessary to consider three main forms for this molecule:

One apolar resonant paramagnetic bi-radical (triplet dioxygen): $^3O_2$ ($^3\Sigma_g^-$):

$^{\bullet a}$:O: —:O:$^{\bullet b}$ ↔ $^{\bullet b}$:O: —:O:$^{\bullet a}$

One polar resonant diamagnetic molecule (singlet dioxygen) $^1O_2$ ($^1\Delta_g$):

:Ö:$^{\ominus}$—:O:$^{\oplus}$ ↔ $^{\oplus}$:O: —:Ö:$^{\ominus}$

One apolar static diamagnetic molecule (singlet dioxygen) $^1O_2$ ($^1\Sigma_g^+$):

:O:=:O:

The Greek symbols in brackets are the rigorous notation using labels derived from the symbols of the irreducible representations of $D_{\infty h}$ point group symmetry. Without such a notation, it would be impossible to distinguish between the two different forms of singlet dioxygen $^1O_2$. This is a crucial point, as these three forms do not have the same energy.

Solving Schrödinger's equation thus shows that the ground state is $^3O_2$ ($^3\Sigma_g^-$) followed by the first excited state $^1O_2$ ($^1\Delta_g$) located at an energy ΔE = 153 zJ (1 zJ = 10^{-21} J) above the ground state. To reach this state, the dioxy-

gen molecule should absorb a photon of wavelength λ = h·c/ΔE, where h is Planck's constant and c the celerity of light in the vacuum. With h·c = 198 645 nm·zJ, the $^1O_2$ ($^1\Delta_g$) state could be reached with a photon of wavelength λ = 198 645/153 = 1298 nm (infrared light). Reaching the second state $^1O_2$ ($^1\Sigma_g^+$), located at an energy ΔE = 259 zJ above the ground state, will involve a photon of wavelength λ = 198 645/259 = 767 nm (red light).

It is then quite unfortunate that most biology textbooks treat dioxygen as a single species, generally written as :O:=:O: or O_2 in short, which is not the ground state formulation. The fact that the spin state (upper left digit before the chemical symbol) is usually not even mentioned is also quite unfortunate. Accordingly, it is worth recalling that spin conservation is one of the most fundamental laws of physics expressed by Witmer-Wigner for chemical transformation[29]. These rules state that if S_A is the spin of reactant A and S_B the spin of reactant B, a reaction will be spin-allowed if the total spin of the products is included in the series: $|S_A + S_B|$, $|S_A + S_B - 1|$, $|S_A + S_B - 2|$,…, $|S_A - S_B|$.

Let us consider, for instance, one of the most exothermic reactions known in chemistry:

$2\ ^1H_2\ (g) + {}^3O_2\ (g) \rightarrow 2\ ^1H_2O\ (l)$
$\Delta_rG° = -2\times237 = -474\ kJ\cdot mol^{-1}$

As indicated by the superscripts showing spin multiplicities, such a direct reaction is spin-forbidden, as we have $S(^1H_2) = ½(1 - 1) = 0$ and $S(^3O_2) = ½(3 - 1) = 1$. It then follows that the total spin of the reactants is S = 2×0 + 1 = 1. For the products, we have $S(^1H_2O) = ½(1 - 1) = 0$, meaning that the total spin of the reactants is S = 2×0 = 0. There is thus a violation of spin conservation in water synthesis from dihydrogen and dioxygen taken in their ground state. This is the reason why nothing happens upon mixing a powerful reductant (H_2) with a powerful oxidant (O_2). However, it is a well-known fact that the reaction is immediate and explosive after the introduction of sparkle in the mixture. The role of sparkle is to bring enough energy to transform triplet oxygen $^3O_2$ ($^3\Sigma_g^-$) into singlet oxygen $^1O_2$ ($^1\Delta_g$). The reaction then becoming:

$2\ ^1H_2\ (g) + {}^1O_2\ (g) \rightarrow 2\ ^1H_2O\ (l)$
$\Delta_rG° = -2\times237 - 95 = -569\ kJ\cdot mol^{-1}$

As now ΔS = 0, the reaction can proceed easily without any catalyst. It is worth recalling here that water synthesis is at the heart of complex-IV (CcO). This complex of the electron transport chain (ETC) in mitochondria has a catalytic site allowing direct reduction of triplet dioxygen into the water using separated fluxes of protons and electrons. The separation of protons from electrons is thus mandatory, as upon mixing them together, we would obtain singlet dihydrogen that is unable to react with triplet dioxygen to form water.

This means that quantum chemistry should be at the heart of biological thinking. The fact that it does not have deleterious consequences, particularly for medicine, as prevention of water synthesis from triplet dioxygen in mitochondria leads to Warburg's effect, a common source for many kinds of diseases[14].

SPECTROSCOPIC PROPERTIES OF SINGLET DIOXYGEN

As explained above, thermal photons may interact with triplet dioxygen ($^3O_2$) to form singlet dioxygen ($^1O_2$). Switch from triplet to singlet state necessitates energy. The most common way to switch to the singlet form is irradiation by visible photon (red at 635 nm), allowing reaching the $^1O_2$ ($^1\Sigma_g^+$) state or infrared ones at 1270 nm, leading to the $^1O_2$ ($^1\Delta_g$) state. The average lifetime of singlet oxygen is 1-50 μs in aqueous systems[30]. In the gas phase, both singlet states may relax towards the triplet state $^3O_2$ ($^3\Sigma_g^-$) by two different mechanisms. The $^1O_2$ ($^1\Delta_g$) state may use collisions with other molecules M according to:

$^1O_2(^1\Delta_g) + n\ M \rightarrow {}^3O_2\ (^3\Sigma_g^-) + n\ M^* + heat$

The notation M* means that, after the collision, the molecules M are left in a rotating state of higher energy. The intrinsic electronic spin has thus been transformed into an extrinsic spin (rotations), ensuring spin-conservation. The generated heat corresponds to the energy difference ΔE = 153 zJ existing between the first excited state and the ground state. The following relationship allows estimating the expected temperature increase ΔT after dissipation of an energy ΔW into heat:

$$\Delta T(K) = \frac{\Delta W(zJ)}{0.0069[6 \times (N - n_C) - 5 \times n_L]}$$

Here, we have used the equipartition theorem of statistical physics $\Delta W = ½k_B\times\Delta T\times\Sigma(df)$, where k_B is Boltzmann's constant and Σ(df), the total number of degrees of freedom concerned by the relaxation process. Now, for a non-linear molecule made of n atoms, one may expect three degrees for the translation of the center of mass, three degrees for the rotation around the center of mass, and 2×(3n – 6) degrees for the normal modes of

vibration. Factor 2 considers that each vibration mode has two degrees, one associated with the position and the second one to speed. Each non-linear molecule will then contribute $6\times½k_B + (3n\text{-}6)\times(½k_B + ½k_B) = ½k_B\times(6n - 6)$. For a linear molecule, the rotation around the molecular axis cannot be used to store energy and thus corresponds to a vibration mode. Each linear molecule will then contribute to $½k_B\times(6n -5)$. It then follows that if n_L stands for the number of linear molecules and n_C for the number of non-linear ones and if N is the total number of atoms, we have $\Sigma(df) = 6\times(N - n_C) - 5\times n_L$.

Water being the most abundant molecule in a cell, we have $N = 3\times n_W$, $n_L = 0$ and $n_C = n_W$, leading to $\Delta T(K) = 12\times\Delta W(zJ)/n_W$. Consequently, in order reaching a temperature T = 310 K (or 37°C) from a temperature T = 288 K (or 15°C, the average temperature of the earth), the total number of concerned water molecules involved in the relaxation of one $^1O_2(^1\Delta_g)$ molecule characterized by ΔW = 153 zJ is estimated as $n_W = 1849/(310 - 288) = 84$ molecules. Furthermore, the average volume v of a molecule having a molecular weight M (Da) in a liquid of density ρ ($g\cdot cm^{-3}$), assuming a random packing efficiency ξ = 0.6366, is given by:

$$V = \xi \times \frac{M \times 10^{-3}}{N_A \times \varrho \times 10^{-3}} \Rightarrow v/Å^3 = 1.057 \times \frac{M(Da)}{\varrho(g \cdot cm^{-3})}$$

For water (M = 18 Da, ρ ≈ 1 $g\cdot cm^{-3}$, i.e. v = 19 $Å^3$), the thermal relaxation volume around one $^1O_2(^1\Delta_g)$ molecule is about $19\times84 = 1598$ $Å^3$, corresponding to a sphere of radius R = 7.3 Å. As the average diameter of isolated water is a molecule is $D = (19\times6/\pi)^{⅓} \approx 3.3$ Å, this corresponds to 2 shells of water molecules. This shows how we may relate a biological number, the average body temperature, to a molecular quantum process relaxation of $^1O_2(^1\Delta_g)$ towards the $^3O_2$ ($^3\Sigma_g^-$) ground state through heating, using well-known physical laws.

Besides this thermal relaxation process involving water molecules, there is a radiative mechanism involving infrared photons:

$$^1O_2(^1\Delta_g) \rightarrow {}^3O_2\ (^3\Sigma_g^-) + {}^3\gamma_{IR}$$

Here, nature uses the fact that a photon is a particle of spin S = 1, allowing photonic relaxation with the emission of a photon spinning in one direction (m_S = +1), leaving the dioxygen molecule in its ground state with the two electrons spinning in the same direction opposite to that of the photon ($m_S = -1/2 - ½ = -1$) to conserve the initial null spin (0 = 1 – 1). Heisenberg's uncertainty relationship drives the timescale associated with such photonic relaxation. It allows relating the intrinsic lifetime τ of the excited state having energy ΔE to the reduced Planck's constant ħ ≈ 106 zJ·fs: ΔE×τ ≈ ħ. Consequently, with ΔE = 153 zJ, it comes that τ ≈ 106/153 = 0.7 fs. This lifetime should be compared with the average rotation time τ_c of a water molecule at a given temperature, needed for allowing thermal non-radiative relaxation. Stokes-Einstein relationship gives this correlation time that depends on absolute temperature T, viscosity η and molecular volume v: $\tau_c = \eta\times v/(k_B\times T)$, i.e. $\tau_c(ps) = 72.4\times\eta(mPa\cdot s)\times v(Å^3)/T(K)$[31]. For liquid water (v = 19 $Å^3$) at T = 310 K, we have η = 0.69 mPa·s, meaning that $\tau_c \approx 3$ ps. This shows that for one molecule undergoing thermal relaxation from the excited state to the ground state, about 5,000 molecules undergo photonic relaxation in the near-IR part of the electromagnetic spectrum.

As the second excited state $^1O_2$ ($^1\Sigma_g^+$) is much higher in energy (ΔE = 259 zJ), its thermal relaxation towards the ground state will mobilize a much more number of water molecules, typically $n_W = 3128/(310 - 288) = 142$ molecules. This forms a relaxation volume of 2,702 $Å^3$, corresponding to a sphere of radius R = 8.6 Å, i.e., nearly 3 shells of water molecules around one $^1O_2$ ($^1\Sigma_g^+$) molecule. The average lifetime of this second excited state being shorter, τ ≈ 106/259 = 0.4 fs, about 7,300 molecules undergo photonic relaxation as a characteristic, red-colored visible light when one relaxes using the thermal channel.

FORMATION OF SINGLET DIOXYGEN

Singlet dioxygen cannot be formed by direct optical excitation of triplet dioxygen by infrared or red photons. Accordingly, from Fermi's Golden rule and group theory, such transitions are both spin-forbidden and orbital-forbidden. This is the reason why a photosensitizer should be used[30]. Another completely different way of forming singlet dioxygen is to use a chemical reaction releasing a large amount of entropy. Reasons for using entropy and not Gibbs' free energies have been analyzed elsewhere[9-11]. Shortly, a single criterion of spontaneous evolution in nature is that entropy of the universe should always increase in any kind of transformation, whether chemical or biological. In other words, biological systems are fully compliant with the second law of thermodynamics with no need to introduce alternate notions such as negentropy, for instance. The observed complexity of biological systems is a consequence of large entropy flux towards the universe, in compliance with the laws of irreversible, far from equilibrium, thermodynamics. From a technical viewpoint to each

transformation of matter corresponds to a change in the standard irreversibility potential $\Delta\pi_i°$ (T = 25°C, p = 1 atm) that cannot be negative. Rules for computing an irreversibility potential $\pi_i°$ for each substance involved in the transformation have been presented elsewhere[10]. For biological systems, such standard irreversibility potentials are transformed to $\pi'_i°$ values considering that biology occurs in water (pH = 7) in the presence of ionic species (ionic strength I ≈ 250 mM). We have used generalized Legendre's transformation, a mathematically straightforward procedure[32]. All the computational details are available as supplementary information (SI).

Irreversibility potentials (IrPs) are useful for comparing two substances according to their entropy content relative to the whole universe. Basically, substances that have strongly negative IrPs are reducing substances. They present a spontaneous tendency to be irreversibly transformed through oxidation into substances having a strongly positive IrP. One may thus notice that singlet dioxygen has a significantly more negative IrP than triplet dioxygen. This automatically means that combustion with $^1O_2$ leads to larger entropy production than combustion with $^3O_2$. Moreover, the burning of a combustible substance existing in a singlet spin-state with $^1O_2$ is spin-allowed, whereas its combustion with $^3O_2$ is spin-forbidden, needing the presence of a catalyst.

In biology, singlet dioxygen plays a key role in photosynthesis. Generation of $^1O_2$ from water molecule has been widely reported during photosynthesis in plants, using energy from the sunlight. Photosensitizers are generally necessary for producing singlet through light absorption. This is particularly true in plants where $^1O_2$ is generated by chlorophyll and other cofactors of the photosystem[33].

In plants exposed to excess light, the increased production of singlet dioxygen can result in cell death[34]. Various substances such as quinones, carotenoids, and tocopherols contained in chloroplasts quench singlet dioxygen and protect against its toxic effects.

In humans, transportation of the dioxygen molecule to the target cell occurs through the triplet state. It is used in the mitochondria together with electrons and protons at the level of the complex IV of the mitochondria, producing water as a non-toxic waste together with some heat and biomolecules with very negative IrPs such as NADH ($\pi_i'° = -6.23829$ zJ·K^{-1}) or NADPH ($\pi_i'° = -1.32422$ zJ·K^{-1}) for instance. It is worth noticing the large difference in irreversibility potentials between NADH and NADPH. However, when considering oxidized forms $NAD^⊕$ ($\pi_i'° = -5.89863$ zJ·K^{-1}) and $NADP^⊕$ ($\pi_i'° = -0.98399$ zJ·K^{-1}), we get almost the same standard oxidation potential:

$NAD^⊕ + H^⊕ + 2\ e^⊖ = NADH \Longrightarrow \Delta\pi_i'° = -0.34534$ zJ·K^{-1} $\Longleftrightarrow E'° = -321$ mV

$NADP^⊕ + H^⊕ + 2\ e^⊖ = NADPH \Longrightarrow \Delta\pi_i'° = -0.34534$ zJ·K^{-1} $\Longleftrightarrow E'° = -321$ mV

Therefore, IrPs are much more useful for biological thinking than oxidation potentials. Accordingly, NADH appears in catabolism for glycolysis, for β-oxidation, by pyruvate dehydrogenase (PDH), by tricarboxylic acid cycle (TCA), in the electron transport chain (ETC), and by nicotinamide nucleotide transhydrogenase (NNT) (35). This simply stems from its negative IrP much lower than any of the non-metallic species. Accordingly, biosynthesis of NADH needs absorption of a large positive entropy flux, such as the one generated at the level of the TCA or the ETC. In deep contrast, NADPH is used in anabolism for performing reductive biosynthesis, in the pentose phosphate pathway (PPP), by isocitrate dehydrogenase (IDP), by the malic enzyme (ME), by aldehyde dehydrogenase (ALDH), and by NADPH-oxidase (36). Owing to its much lower IrP, biosynthesis of NADPH needs a much smaller positive entropy flux than the one required for NADH. It follows that NADPH is more able to drive biosynthetic pathways and is also involved in redox sensing and as a substrate of NADPH oxidases for generating reactive oxygen species. So, we have here a good example of two remarkably similar reductants having quite contrasted entropy content, explaining the observed strong compartmentalization of redox functions in a living cell.

It is also worth noticing that at the mitochondrion level, there is the orientation of the positive entropy flux towards the synthesis of biomolecules displaying large positive IrPs. Such molecules play the role of "canned entropy" for driving molecular machines, just like batteries act as "canned electricity" for driving electrical motors. The best candidates are polyphosphates such as adenosine diphosphate (ADP with $\pi_i'° = +7.93486$ zJ·K^{-1}) or adenosine triphosphate (ATP with $\pi_i'° = +12.76803$ zJ·K^{-1}). Accordingly, the positive entropy flux for making ATP from ADP appears too small relative to their entropy content:

$ADP + P_i = ATP + H_2O \Longrightarrow \Delta\pi_i'° = -0.2007$ zJ·K^{-1} $\Longleftrightarrow$ pK = 6.3

At a temperature T = 298.15 K, such a pK-value corresponds to a free energy change $\Delta G' = +36.0$ kJ·mol^{-1} = 60 zJ. Conversely, this is just the amount of heat that would be generated upon the hydrolysis of ATP into ADP. Here, it is worth using our relationship $\Delta T(K) \approx 12\times W(zJ)/n_W$ allowing converting an amount of heat W into a temperature change ΔT after spreading such heat

among n_W water molecules. Choosing ΔT = 1 K for W = 60 zJ leads to n_W = 720 or $R_W = 1.66 \times n_W^{1/3}$ = 14.9 Å, in terms of radius of the hydration shell surrounding the spatial location of the reaction. Now, on average, four shells of water molecules surround each biomolecule in a living cell[37]. This translates into a radius of hydration $R_h = 4 \times 3.3$ = 13.2 Å, a value close to the radius of conversion of entropy into heat R_W. As explained in previous papers (9,10), the main role of ATP in a living cell is not to provide energy but rather to play the role of a powerful hydrotrope[35]. ATP has thus the crucial double role of being both an entropy sink and avoids by its presence the irreversible coagulation of proteins.

There is obviously not enough entropy liberated through hydrolysis of a single ATP molecule to convert triplet dioxygen into singlet dioxygen. From the relative IrPs of $^1O_2$ and $^3O_2$ and with $\Delta\pi_i'^\circ$ = 0.2007 zJ·K^{-1} for ATP hydrolysis, the formation of singlet dioxygen from triplet dioxygen would require the simultaneous hydrolysis of at least n(ATP) = sup(0.53256/0.2007) = 3 molecules. As this is very unlikely on the statistical ground or as it would involve a huge protein, it may seem that singlet dioxygen would have a negligible role to play in a living cell favoring triplet dioxygen. This is, of course, the conventional biological thinking putting the exclusive focus on the ground state $^3O_2$ ($^3\Sigma_g^-$) with very few references to the first excited state $^1O_2$($^1\Delta_g$). Owing to its quite negative IrP, very few substances can create singlet dioxygen as a waste. Among them, we have, for instance, ozone $^1O_3$. It is easy checking that water has entropy high enough to resist oxidation into hydrogen peroxide by ozone:

$^1O_3 + {}^1H_2O = {}^1O_2 + {}^1H_2O_2$
$\Delta\pi_i'^\circ$ = -0.22868 zJ·K^{-1} ⟺ pK = 7.2

This is not the case of hydrogen peroxide that is easily reduced into the water by ozone with singlet dioxygen as a by-product:

$^1O_3 + {}^1H_2O_2 = 2\ {}^1O_2 + {}^1H_2O$
$\Delta\pi_i'^\circ$ = +0.29663 zJ·K^{-1} ⟺ pK = -9.3

Suppose the reaction leading to triplet dioxygen is much more favorable, it is, however, spin-forbidden, allowing singlet dioxygen to be the main kinetic product in the absence of a catalyst. The trouble is that if ozone is an important compound in the atmosphere owing to its irradiation by the sun, its occurrence in a living cell is not so obvious.

Singlet dioxygen may also be produced in a living cell subjected to an oxidative stress upon annihilation of oxygen-based radicals:

$^2O_2^{\bullet\ominus} + {}^2OH^\bullet = {}^1O_2 + {}^1OH^\ominus$
$\Delta\pi_i'^\circ$ = +0.80038 zJ·K^{-1} ⟺ pK = -25

$^2HO_2^\bullet + {}^2HO_2^\bullet = {}^1O_2 + {}^1H_2O_2$
$\Delta\pi_i'^\circ$ = +0.03602 zJ·K^{-1} ⟺ pK = -1.1

However, such reactions are in competition with formation of triplet dioxygen and a much larger entropy release:

$^2O_2^{\bullet\ominus} + {}^2OH^\bullet = {}^3O_2 + {}^1OH^\ominus$
$\Delta\pi_i'^\circ$ = +1.33282 zJ·K^{-1} ⟺ pK = -42

$^2HO_2^\bullet + {}^2HO_2^\bullet = {}^3O_2 + {}^1H_2O_2$
$\Delta\pi_i'^\circ$ = 0.56846 zJ·K^{-1} ⟺ pK = -18

Catalysis of this last reaction *in vivo* involves the well-studied enzyme superoxide dismutase (SOD) that is not affected by the presence of singlet oxygen[35].

As triplet dioxygen has higher irreversibility potential than singlet dioxygen, it will always play the role of the thermodynamically favored species. This means that the production of singlet dioxygen using the annihilation of inorganic radicals is, as a rule, quite difficult to control. This is no more the case by using singlet species, as even if the formation of triplet dioxygen is still more favorable, it becomes slow as the reaction is now spin-forbidden. Here is a good example that readily occurs in neutrophils, for instance:

$^1ClO^\ominus + {}^1ClO^\ominus = {}^1O_2 + 2\ {}^1Cl^\ominus$
$\Delta\pi_i'^\circ$ = +0.39840 zJ·K^{-1} ⟺ pK = -12.5

However, such a reaction requires a high concentration of the rather unstable hypochlorous ion. This is the reason for the extensive use of phagosomes by neutrophils. Under diluted conditions, there is the possibility of using hydrogen peroxide, forming as by-products water and chloride ions:

$^1H_2O_2 + {}^1ClO^\ominus = {}^1O_2 + {}^1Cl^\ominus + {}^1H_2O$
$\Delta\pi_i'^\circ$ = +0.46186 zJ·K^{-1} ⟺ pK = -14.5

It is worth noting that use of the hypochlorous ion is mandatory, as the entropy difference between the chlorous and hypochlorous species is not high enough for allowing the production of singlet dioxygen:

$^1H_2O_2 + {}^1ClO_3^\ominus = {}^1O_2 + {}^1ClO_2^\ominus + {}^1H_2O$
$\Delta\pi_i'^\circ$ = -0.09802 zJ·K^{-1} ⟺ pK = 3.1

TRAPPING OF SINGLET DIOXYGEN

Singlet dioxygen could be very harmful to normal cells. The first reason stems from the fact that there is no spin restriction for reacting with other singlet molecules. A second reason is that it has a quite negative IrP. It is worth recalling here its Lewis' structure:

$$:\ddot{O}:^{\ominus}—:O:^{\oplus} \quad \leftrightarrow \quad {}^{\oplus}:O:—:\ddot{O}:^{\ominus}$$

A most prominent feature is the formal positive charge on one of the two oxygen atoms, meaning that singlet dioxygen has a high affinity for any electron-rich centers. Among them, carbon atoms engaged in a C=C double bond are sites for preferential attack owing to their complementary dynamic Lewis' structure:

$$>C=C< \quad \leftrightarrow \quad >C:^{\ominus}—C^{\oplus}< \quad \leftrightarrow \quad >{}^{\oplus}C—C:^{\ominus}<$$

The reaction of singlet dioxygen with C=C double bonds often leads to the formation of endoperoxides (figure 1).

For a single C=C double bond, the resulting endoperoxides have a quite strained four-membered ring, leading to a highly unstable addition compound. This is not the case when oxidation leads to a rather stable six-membered ring, a situation encountered in any molecule containing at least two conjugated C=C double bonds.

Singlet dioxygen may react rapidly with other singlet molecules forming species such as hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2) or superoxide radical ($^{\bullet}O_2^-$). These reactive oxygen species will oxidize DNA (mutation and DNA breaks), proteins and lipids. Here is a list of favorable reactions with ubiquinol (H_2CoQ_{10}), ascorbic acid (vitamin C, $AscH_2$), reduced cytochrome-c, dihydrolipoic acid (DHLA), reduced glutathione (GSH) and free iron (II):

$$^1O_2 + {}^1H_2CoQ_{10} = {}^1H_2O_2 + {}^1CoQ_{10}$$
$$\Delta\pi_i{}'^{\circ} = +0.850\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -27$$

$$^1O_2 + {}^1AscH_2 = 2\ {}^2HO^{\bullet} + {}^1DHA$$
$$\Delta\pi_i{}'^{\circ} = +0.680\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -21$$

$$^1O_2 + {}^1AscH_2 = {}^1H_2O_2 + {}^1DHA$$
$$\Delta\pi_i{}'^{\circ} = +1.719\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -54$$

$$^1O_2 + \text{cyto-}{}^1Fe^{2\oplus} = {}^2O_2{}^{\bullet\ominus} + \text{cyto-}{}^2Fe^{3\oplus}$$
$$\Delta\pi_i{}'^{\circ} = +0.326\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -10$$

$$^1O_2 + {}^1DHLA = 2\ {}^2HO^{\bullet} + {}^1ALA$$
$$\Delta\pi_i{}'^{\circ} = +0.195\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -6$$

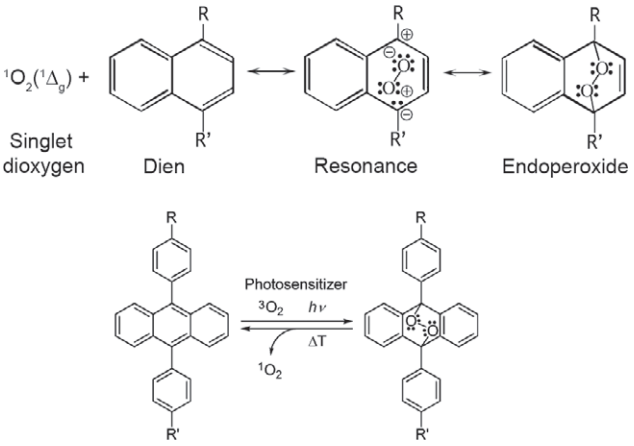

Figure 1. Affinity of singlet dioxygen for conjugated double bonds leading to the formation of an endoperoxide bridge. Endoperoxides may also be formed from triplet dioxygen in the presence of a photosensitizer. One may speak of endoperoxides as "canned singlet dioxygen" owing to their ability to release $^1O_2$ upon heating.

$$^1O_2 + 2\ {}^1GSH = 2\ {}^2HO^{\bullet} + {}^1GSSG$$
$$\Delta\pi_i{}'^{\circ} = +0.173\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -5$$

$$^1O_2 + Fe^{2\oplus} + = {}^2O_2{}^{\bullet\ominus} + {}^2Fe^{3\oplus}$$
$$\Delta\pi_i{}'^{\circ} = +0.049\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = -2$$

It is worth noticing the mandatory generation of hydrogen peroxide with ubiquinol, as there is, in this case, not enough entropy for generating two hydroxyl radicals:

$$^1O_2 + {}^1H_2CoQ_{10} = 2\ {}^2HO^{\bullet} + {}^1CoQ_{10}$$
$$\Delta\pi_i{}'^{\circ} = -0.190\ zJ{\cdot}K^{-1} \Longleftrightarrow pK = 6$$

SINGLET DIOXYGEN, OZONE, AND RADIATION THERAPY

The above reactions explain why singlet oxygen ($^1O_2$) is widely used in photodynamic therapy of cancer. During photodynamic therapy, photosensitizers excited by light react with ground state oxygen $^3O_2$, which leads to the generation of this major cytotoxic agent. After generation, singlet dioxygen oxidizes all the molecules responsible for the redox homeostasis of the cell rapidly, killing the surrounding tissues and cells[38].

It has been more than 60 years since the discovery of the oxygen effect that empirically demonstrates the direct association between cell radiosensitivity and oxygen tension, important parameters in radiotherapy. However, no real understanding of the mechanisms underlying this principle tenet of radiobiology is yet available[39].

Photons react with water to form free radicals, including singlet oxygen. Singlet oxygen interacts with the mitochondria to cause the permeabilization of the mitochondrial outer membrane, leading to the cytosolic release of pro-apoptotic proteins and to the impairment of the bioenergetic functions of mitochondria and resulting apoptosis[40].

About twenty years ago, it was shown by Wentworth *et al.* that antibodies catalyze the generation of ozone by a water oxidation pathway[41]. It was first postulated that dihydrogen trioxide [H_2O_3] was a key intermediate. However the direct formation of this intermediate is not thermodynamically favorable:

$$^1O_2 + {}^1H_2O = {}^1H_2O_3$$
$$\Delta\pi_i'^\circ = -0.509\ zJ\cdot K^{-1} \Longleftrightarrow pK = 16$$

It is worth noticing that adding another singlet dioxygen cannot oxidize water into ozone O_3 according to:

$$2\ {}^1O_2 + {}^1H_2O = {}^1O_3 + {}^1H_2O_2$$
$$\Delta\pi_i'^\circ = -0.297\ zJ\cdot K^{-1} \Longleftrightarrow pK = 9$$

However, upon generation of at least three singlet dioxygen molecules, water oxidation becomes possible with the release of triplet dioxygen as waste:

$$3\ {}^1O_2 + {}^1H_2O = {}^1O_3 + {}^1H_2O_2 + {}^3O_2(aq)$$
$$\Delta\pi_i'^\circ = +0.236\ zJ\cdot K^{-1} \Longleftrightarrow pK = -7$$

However, such a reaction is spin-forbidden. Hence, we propose this final scheme, which is spin-allowed:

$$4\ {}^1O_2 + {}^1H_2O = {}^1O_3 + {}^1H_2O_2 + 2\ {}^3O_2(aq)$$
$$\Delta\pi_i'^\circ = +0.768\ zJ\cdot K^{-1} \Longleftrightarrow pK = -24$$

Owing to the liberation of ozone, any tumor would be burnt with the generation of only gases as wastes. Moreover, one of the reactants is the water molecule, the most abundant chemical species in a living cell. The crucial point is that water no more acts here as a solvent whose activity is equal to one, owing to its huge abundance. It is a well-established fact that the status of water in tumors is quite different from that of water in a normal cell. In thermodynamics language, this translates into the fact that water activity cannot be the same in a tumor and in a normal cell[42–46]. As the above equilibrium is sensitive to water activity, one may expect different yields of ozone according to the status of water in the cell exposed to radiations able to generate singlet dioxygen in the large amount. In other words, there is a possibility of targeting any $^1O_2$-treatment towards cancer cells, leaving normal cells relatively unaffected.

The radiation therapist knows that soft tumors like lymphomas and seminoma are more sensitive to radiation than harder ones. Accordingly, doses needed to eradicate seminoma and lymphoma is smaller, and the treatment is shorter than the treatment of squamous cell carcinoma or adenocarcinoma. The earlier sign of tumor response during radiation therapy is the change of consistency (harshness) of the tumor. This is in line with a change in the activity of water (see above).

CONCLUSION

It is possible that ionizing radiation such as produced by modern linear accelerators act at the cellular level by the mean of thermal photons. These photons will induce, in turn, the synthesis of singlet dioxygen. In such a scheme of thought, high-energy photons are just a way to deliver thermal photons to deep-seated tumors. Infrared photons are not powerful enough to reach these lesions. Absorption of over 90% of the dose occurs in the first cm[47].

Cytotoxic chemotherapy activates the concentration of free radicals such as the ones induced by singlet dioxygen or radiation therapy. This is evident by the elevation of lipid peroxidation products; the reduction in plasma levels of antioxidants such as vitamin E, vitamin C, and β-carotene; and the marked reduction of tissue glutathione levels that occurs during chemotherapy. Those agents that generate high levels of ROS include the anthracyclines (e.g., Doxorubicin, Epirubicin, and Daunorubicin), alkylating agents, platinum coordination complexes (e.g., Cisplatin, Carboplatin, and Oxaliplatin), epipodophyllotoxins (e.g., Etoposide and Teniposide), and the Camptothecins (e.g., Topotecan and Irinotecan) [48]. One other option to improve the efficacy of infrared photons is to activate a photosensitizer such as methylene blue[49].

Moreover, an often-overlooked fact is that water activity is higher in cancer cells than in normal cells. As demonstrated just above this could mean that in a cancer cell, singlet dioxygen may react with water yielding ozone, a powerful oxidant. Such a possibility opens the road to a non-linear hormetic behavior of singlet dioxygen. Typically, we expect a harmful increase of oxidative stress at low concentration, a healing effect against cancer at moderate concentration (due to selective in-situ formation of ozone) and a well-documented cytotoxic effect towards any kind of cell at high concentration. Future experimental research is needed to confirm

or reject such a putative behavior suggested by available thermodynamic data.

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ANNEX

Table 1 gives irreversibility potentials (IrPs or π_i'°) values in ascending order for species discussed in this work. As the whole universe is by definition a closed system, this allows, in compliance with the second law, to identify three kinds of processes in nature:

i) Irreversible processes that are spontaneous being such that $\Delta\pi'_i{}^\circ > 0$.
ii) Fully reversible processes are characterizing equilibrium situations as $\Delta\pi'_i{}^\circ = 0$.
iii) Non-spontaneous processes, such that $\Delta\pi'_i{}^\circ < 0$, thus requiring to be coupled with another spontaneous process characterized by $\Delta\Pi_i'^\circ > -\Delta\pi'_i{}^\circ > 0$.

Moreover, owing to their definition, irreversibility potentials changes may be related to equilibrium constants K, or to standard oxidation potentials E'°, using the following conversion relationships (T = 298.15 K):

$$\begin{cases} pK = -log_{10}K = -31.456 \times \Delta\pi_i'^\circ(zJ \cdot K^{-1}) \\ E^\circ(V) = \dfrac{1.860906}{n} \times \Delta\pi_i'^\circ(zJ \cdot K^{-1}) \end{cases}$$

Conversion into standard oxidant potentials are for transformations involving electrons and requires the knowledge of the number of electrons n that should be added to an oxidant to transform such species into its conjugated reduced form.

Let us consider for instance the two-electrons reduction of protons into dihydrogen ($2\ H^{\oplus} + 2\ e^{\ominus} = H_2$) or the four-electrons reduction of dioxygen into water ($^3O_2 + 4\ H^{\oplus} + 4\ e^{\ominus} = 2\ H_2O$). From table 1, we evaluate that:

$$\begin{cases} E'^{\circ}(H^{\oplus}/H_2) = \dfrac{-1.860906 \times 0.55211}{2} = -0.51\ V \\ E'^{\circ}(O_2/H_2O) = \dfrac{1.860906 \times (2 \times 0.86694 + 0.09134)}{4} = +0.85\ V \end{cases}$$

This allows classifying dihydrogen as a reductant ($E'^{\circ} < 0$) and dihydrogen as an oxidant ($E'^{\circ} > 0$). But, one may also consider reacting dihydrogen with dioxygen in order to produce water ($2\ H_2 + O_2 = 2\ H_2O$). Electrons being eliminated, the irreversibility potential change is now expressed as equilibrium constant K:

$$pK = -31.456 \times [2 \times (0.8667 + 0.55218) + 0.09135) = -92.1$$

As at T = 298.15K, we have $\Delta G'^{\circ}(kJ{\cdot}mol^{-1}) = RT{\cdot}\ln(10){\times}pK = 5.708{\times}pK$, the highly positive $\Delta\pi_i'^{\circ} = 2.92943\ zJ{\cdot}K^{-1}$ variation responsible to the quite negative pK, corresponds to a large negative change of the so-called "Gibbs' free energy," viz. $\Delta G'^{\circ} = -526\ kJ{\cdot}mol^{-1}$. With such a pK value, one may conclude that water synthesis is a spontaneous quasi-quantitative process. The reason for such a huge release of entropy is obvious after noticing that on the right of the equation, a substance with a large positive irreversibility potential appears, whereas, on the left, two substances with negative irreversibility potentials disappear.

Leading the left column, we find species with large negative potentials (reductants), thus providing the largest entropy production upon their transformation into species located on the right column (oxidized forms). Consequently, such species are to be considered as useful low entropy "food." Reciprocally, species at the bottom of the right column are generally end products in a chemical transformation, owing to their high entropy content. Consequently, they may be qualified as "waste" that will be eliminated in order to maintain the largest entropy gradient in the living organism. Another crucial point is that we find in both columns radical species holding unpaired electrons. This means that some radicals should be considered as food and others as waste. Moreover, some radicals may be strong reductants, such as atomic hydrogen:

$$H^{\oplus} + e^{\ominus} = H^{\bullet}(aq) \Longrightarrow \Delta\pi_i'^{\circ} = -1.46898\ zJ{\cdot}K^{-1} \Longleftrightarrow E'^{\circ} = -2.31\ V$$

On the other hand, the hydroxyl radical $HO^{\bullet}$ behaves as a strong oxidant:

$$HO^{\bullet} + H^{\oplus} + e^{\ominus} = H_2O \Longrightarrow \Delta\pi_i'^{\circ} = +1.24046\ zJ{\cdot}K^{-1} \Longleftrightarrow E'^{\circ} = +2.73\ V$$

Table 1. Irreversibility potentials $\pi_i'^{\circ}$ and corresponding standard free energies of formation $\Delta_fG'^{\circ}$ for chemical species considered in this work. Values computed at T = 398.15 K, pH = 7 for an ionic strength I = 0.25 M.

Species	$\pi_i'^{0}$/zJ·K-1	$\Delta_fG'^{\circ}$/zJ	$\Delta_fG'^{\circ}$/kJ·mol^{-1}
CoQ10	-25.28740	7539	4540.36
$CoQ10H_2$	-25.22108	7520	4528.45
DHLA	-1.77480	529	318.67
$H^{\bullet}$(aq)	-1.46898	438	263.75
ALA	-1.45613	434	261.45
O_3	-0.96965	289	174.10
$^1O_2$	-0.62378	186	112.00
H_2(aq)	-0.55211	165	99.13
$^1O_2$(g)	-0.52665	157	94.56
H_2(g)	-0.45409	135	81.53
$HO^{\bullet}$	-0.37352	111	67.07
H_2O_3 (C_2-symmetry)	-0.26618	79	47.79
$HO_2^{\bullet}/O_2^{\bullet\ominus}$	-0.18370	55	32.98
$^3O_2$(aq)	-0.09134	27	16.40
$^3O_2$(g)	-0.00000	0	0.00
Cytc-[$Fe^{3\oplus}$]	0.04059	-12	-7.29
$[ClO_3]^{\ominus}$	0.04879	-15	-8.76
$Fe^{3\oplus}$(aq)	0.06676	-20	-11.99
Cytc-[$Fe^{3\oplus}$]	0.15455	-46	-27.75
$HOCl/ClO^{\ominus}$	0.22429	-67	-40.27
H_2O_2	0.29239	-87	-52.50
$Fe^{2\oplus}$(aq)	0.45747	-136	-82.14
$Cl^{\ominus}$	0.73538	-219	-132.04
H_2O	0.86694	-258	-155.66
GSH	1.52051	-453	-273.01
$AscH_2$	3.03142	-904	-544.29
GSSG	3.33710	-995	-599.18
DHA	3.83413	-1143	-688.42
P_i	5.90080	-1759	-1059.49
NADH	6.10589	-1820	-1096.31
$NAD^{\oplus}$	6.44221	-1921	-1156.70
ADP	7.93486	-2366	-1424.71
NADPH	11.08026	-3304	-1989.46
$NADP^{\oplus}$	11.40756	-3401	-2048.23
ATP	12.76803	-3807	-2292.50

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Research Articles

Is the Second Law of Thermodynamics Able to Classify Drugs?

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Abstract. Specialization characterizes pharmacology, with the consequence of classifying the various treatments into unrelated categories. Treating a specific disease usually requires the design of a specific drug. The second law of thermodynamics is the driving force both for chemical reactions and for life. It applies to diseases and treatment. In most common diseases, there is a metabolic shift toward anabolism and anaerobic glycolysis, resulting in the release of entropy in the form of biomass. In accordance with the second principle of thermodynamics, treatment should aim at decreasing the entropy flux, which stays inside the body in the form of biomass. Most treatments aim at increasing the amount of entropy that is released by the cell in the form of thermal photons. As clinically different diseases often requires similar drugs, this calls for reinforcement in a quest for a single unified framework. For example, treatment of aggressive autoimmune diseases requires the same cytotoxic chemotherapy than for cancer. This strongly suggests that despite their apparent disparity, there is an underlying unity in the diseases and the treatments. The shift toward increased entropy release in the form of heat offers sound guidelines for the repurposing of drugs.

Keywords: Pharmacology, Alzheimer, psychiatry, cancer, entropy, pHi, mitochondria, lactic acid, paradigm shift.

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1. INTRODUCTION

This paper is one of a series of publications trying to merge medicine back into physics. Accordingly, the combination of theory and subsequent experiments was the cause of major progress in physics. We aim at describing diseases and thus treatment as physical features. But in medicine, measurements of physical data such as calculation of entropy are missing. Entropy production and dissipation has never been measured in human cells. We are, at a stage, where we can only raise hypothesis based of indirect markers of the fluctuation of entropy.

We have based our reasoning on data based on the metabolic flux centered by the mitochondria, the place within a cell, providing the maximum production of entropy as heat. We also have used clinical data. For example, if the patient is more active (like after treatment with thyroid hormones) one can assume that the entropy flux has increased. Similarly, if the temperature decreases (like after antibiotic treatment for infection) one may deduce that there is a decrease in entropy released in the outer space. In this paper, we have tried to merge biological and medical data, focusing on their impact on entropy. The second law of thermodynamics tells us that entropy can only increase in a closed system. When discussing the second law of thermodynamics, one should always define its reference. Here we consider the human body as the reference point. The entropy can be excreted from the patient and thus locally decreases. But, the entropy of the universe is always going up, as the contribution from our body, compared to the Sun-Earth system, is almost negligible.

2. BACKGROUND

In the vast majority of diseases, there is a shift toward increased synthesis of biomass. In cancer, there is increase in cellular proliferation. In neurodegenerative diseases, there are protein deposits like the amyloid plaques in Alzheimer's disease or the bodies of Lewy in Parkinson's disease. In inflammation, there is secretion of proteins such as lymphokines and cytokines and proliferation of inflammatory cells.

The Nobel Prize, Otto Warburg (1883-1970) in the 1920s, first described this shift toward anabolism in cancer cells. The Warburg's effect is a modified cellular metabolism based on aerobic fermentation, which tends to favor anaerobic glycolysis rather than oxidative phosphorylation, even in the presence of oxygen.

In epithelial cells, the Warburg's effect results in cancer[1]. The Warburg's effect is a bottleneck. The cells cannot burn the glucose because the pyruvate cannot be degraded in the Krebs' cycle. Evidence of the central role of the Warburg's effect comes when the researcher injects into cancer cells, with a micropipette, normal mitochondria. The growth will stop. These cells have become benign. The injection of the nuclei of cancer cells into normal cells does not increase growth. These cells can still burn glucose because the mitochondria are normal and do not form tumors [1 and references therein]. The inhibition of the oxidative phosphorylation results in the activation of the anabolic pathway, such as the Pentose Phosphate Pathway (PPP), that is necessary for DNA and RNA synthesis [1 and references therein].

The Warburg's effect results in the release of lactic acid in the extracellular space, the concomitant activation of the Pentose Phosphate Pathway, and anabolism[1]. The Warburg's effect results in the synthesis of new proliferating cells[1]. More recently, metabolic shifts have been described in Alzheimer and Parkinson's diseases[2,3]. Similar shifts toward anaerobic glycolysis have been described in most common disease. To name a few, among others, as published in[4]: autism[5,6] schizophrenia[7], Alzheimer[3], Parkinson's disease[8,9] Huntington's disease[10], stroke[11,12], infection[13], fibrosis[14,15], cirrhosis[16,17], emphysema[18], arthritis[19], scleroderma[20], lupus[21,22]. To the difference to the Warburg effect, these shifts toward glycolysis and increased lactate secretion may be transient and reversible in the presence of oxygen[4].

In biology, like in physics and chemistry, we are dealing with intertwined variables. In physics, Newton's law links forces to momenta. In chemistry, the ideal gas law links pressure, volume, temperature and the amount of matter. In biology, the release of entropy in the form of heat, oxidative phosphorylation, high mitochondrial activity and acidic intracellular pHi are linked if not synonymous. It seems that there is a shift to mitochondrial impairment in almost every disease, resulting in increased lactate concentration. These diseases appear to be a consequence of impaired mitochondrial function and increased entropy release in the form of biomass.

In cancer, mitochondrial impairment results in cell proliferation and tumor growth. In Alzheimer disease, there is an abnormal secretion of amyloid plaques, in Parkinson's disease, there are intracellular deposits (Lewy bodies) [3 and references therein].

A basic equation for cellular life taking into account that cells are open systems have been previously proposed[4,23-25]:

$$\text{foods (in)} = \text{biomass (in)} + \text{heat (out)} + \text{wastes (out)} \quad (1)$$

where "in" and "out" refer to a system surrounded by a containment able to exchange heat and matter between the inside and the outside of the cell.

Catabolism powered by oxidative phosphorylation is another word for entropy release in the form of heat. Anabolism through fermentation is another name for entropy release in the form of biomass[24].

Thus, from a thermodynamic standpoint, diseases can be classified according to the second law of thermodynamics. The cell feeds on low entropy molecules such as glucose to release higher entropy molecules such as CO_2 and ATP[23]. To comply with the second law of ther-

modynamics, the cell absorbs and degrades low entropy compounds into heat or biomass with a neat production of entropy[24].

Differentiated cells release their entropy in the form of thermal photons[25]. Proliferating cells have lower mitochondrial activity and release their entropy in the form of biomass. Differentiated cells have an increased mitochondrial activity [26–29], resulting in the release of entropy in the form of heat. Differentiated cells have a basal oxidative metabolism. The efficient TCA cycle degrades glucose into pyruvate [1,30]. The oxidative phosphorylation of acetyl-CoA into mitochondria yields large amounts of entropy-rich ATP and releases carbon dioxide and water as entropy-rich waste products.

This is the opposite of proliferative cells. Biomass synthesis and cell growth requires a rewiring of the carbon flux. Here, the PPP acts as a shunt for glycolysis, generating nucleic acid precursors for DNA replication [1,30]. Poorly differentiated cells release their entropy in the form of biomass[25]. Undifferentiated cells have lower mitochondrial activity resulting in alkaline pH and lower transmembrane potential, and increased cell division[31].

Cells oscillate between two modes of entropy production. Differentiated cells release entropy in the form of heat. They have high ATP production, increased transmembrane potential, increased ionic concentration, intracellular acidic pH, and low water activity. On the other hand, proliferative cells have decreased ATP synthesis, diluted ionic content, low transmembrane potential, alkaline pH[26].

During adulthood, respiration is predominant[32]. Childhood and aging are more anabolic than adulthood. In childhood, anabolism results mostly in growth. In aging, anabolism results in age-related diseases such as cancer and Alzheimer's disease. Most drugs impact both anabolism and catabolism. For clarity, we will focus on what appears to be the main target of the drug.

3. ATTEMPT OF CLASSIFICATION

Diseases can be described as a perturbation of the flux of entropy[24]. Most drugs should be described based on their impact on the entropy flux. Drugs have been designed to target a specific receptor. As a key opens the lock, the active compound binds with its receptor and modify the fate of the targeted cell. This view of pharmacology has yielded tremendous results, most recently, with the advent of targeted therapies. Our goal is to complement this approach with the necessary compliance to the second law of thermodynamics. But most drugs have multiple effects on entropy. For example, as we will discuss later, thyroid hormones increase entropy production, decrease the release of entropy in the form of biomass and shift toward the release of entropy in the form of thermal photons.

A) Drugs increasing the release of entropy

Some diseases are a consequence of a decreased metabolic activity, resulting in decreased production of entropy. A decrease in physical and psychological activity is the hallmark of these diseases. In hypothyroidism, there is constipation, somnolence and sometimes depression[33].

Treatment with thyroid hormones results in increased heart rate, weight loss and excitability[34]. Thyroid hormones result in increased entropy flux, but also in a shift toward increased release of thermal photons. Overdose of thyroid hormones may result in hyperactivity, fever, mania, diarrhea and increased heart rate. These are indirect signs of increased production of entropy.

The mechanism underlying the regulation of the basal metabolic rate by thyroid hormones remains unclear. It has been suggested that these hormones uncouple substrate oxidation from ATP synthesis. A molecular determinant of the effects of T3 could be uncoupling protein-3 (UCP-3)[34]. Such uncoupling from ATP synthesis results in increased secretion of heat in the form of thermal photons[35].

Amphetamines are a class of psychotropic drugs with high abuse potential, as a result of their stimulant, euphoric and hallucinogenic properties. Amphetamines are synthetic drugs, of which methamphetamine, amphetamine, and 3,4-methylenedioxymethamphetamine ("ecstasy") represent well-recognized examples. Resulting from their amphiphilic nature, these drugs can easily cross the blood–brain barrier and elicit their well-known psychotropic effects[36]. Both cocaine and amphetamine induces the secretion of uncoupling protein, resulting in thermogenesis[37].

Cardiac stents reestablishes the blood flow to the diseased heart and peripheral tissues, resulting in the restoration of the metabolism and increased entropy production. Antiarrhythmic and cardio tonics increase the efficacy and improve the contraction of the heart, which leads to increased quantity of blood flow to the peripheral tissues. It results an improvement of metabolism and increase production of entropy and a better well being of the patient.

The drugs aiming at treating cardiovascular diseases are numerous. They, all, aim at increasing the efficacy of

the cardiac pump. Digoxin intensifies the phosphorylating activity of mitochondria[38]. Digoxin stimulates the mitochondrial activity and thus decreases the amount of entropy released in the form of biomass. Digoxin has been repurposed in the treatment of cancer[39]. The addition of oxygen to patients with cardiac or pulmonary failure results in better mitochondrial efficacy and enhanced synthesis of thermal photons[40].

Table 1. Drugs and devices increasing entropy production.

Thyroid hormones[34,35]
Amphetamines[36,37]
Cocaine[37]
Cardiac stents
Digoxin[39,39]
Oxygen[40]

B) Drugs decreasing the flux of entropy

Caloric restriction, without malnutrition, delays aging[41] and extends life span in diverse species including humans. Caloric restriction delays the onset of age-associated pathologies. Specifically, caloric restriction reduces the incidence of diabetes, cancer, cardiovascular disease and Alzheimer's disease[41].

The brain has the highest energy consumption of the body (around 20% of the body oxygen and 25% of the glucose) while representing 3% of our body's mass. Biological conditions, which decrease mitochondrial energy yield, would impact brain functions, increasing vulnerability to brain disorders[42].

Drugs, which decrease the metabolism, slow down the flux of entropy. Anesthetics such as lidocaine, an amide local anesthetic, decreases glucose uptake through the reduction of the expression of GLUT1 and HK2. Lidocaine inhibits the enhanced glycolysis and glycolytic capacity induced by LPS in the macrophages [43,44]. Similarly, halothane anesthesia decreases glucose uptake because of transient inhibition of brain phosphofructokinase[45].

Intraperitoneal injections of phenobarbital decrease the concentration of glucose-6-phosphate and fructose-6-phosphate and result in the depression of the motor activity. The finding of decreased hexose phosphates in the brain supports the hypothesis that central depressant drugs suppress glycolysis in the central nervous system in vivo possibly by a diminution of glucose phosphorylation[46].

Propofol is another of the most commonly used sedative. This drug inhibits anaerobic glycolysis and the Krebs' cycle. Consistently, propofol inhibited the expression and glycolysis proteins (GLUT1, HK2 and LDHA)[47].

Conventional medications against seizure reduce neuronal excitability through effects on ion channels or synaptic function. Recently, it has become clear that metabolic factors also play a crucial role in the modulation of neuronal excitability[48]. In 1955, Greengard[49] demonstrated that anticonvulsant prevents the rise in oxidation such as seen during seizures. The clinical effectiveness of a variety of diets based on metabolism, especially for children with epilepsy refractory to medication, underscores the applicability of metabolic approaches to the control of seizures and epilepsy. Such diets include the ketogenic diet. A promising avenue to alter cellular metabolism, and hence excitability, is by partial inhibition of glycolysis, which has been shown to reduce seizure susceptibility in a variety of animal models as well as in cellular systems in vitro. One such glycolytic inhibitor, 2-deoxy-d-glucose (2DG), increases seizure threshold in vivo and reduces interictal and ictal epileptiform discharges[50].

Anxiety could be viewed as a shift toward the transient Warburg effect. An argument for a mitochondrial explanation of anxiety is stress's capacity to trigger the shift toward a decreased energy yield of the mitochondria. For example, catecholamines induce Warburg's effect and the secretion of lactate[51]. In times of stress, catecholamines can bind muscle cell receptors and trigger the breakdown of glycogen to lactate, diffusing out into circulation and used as a fuel. Similarly, hypoxia is a risk factor for anxiety[52]. Hyperventilation is a cause for hypoxia, which leads to anxiety and panic attacks[53]. Sleep apnea causes a panic attack[54]. In 1967, Pitts and McClure suggested that a raised lactate level in blood and body fluids causes all symptoms of anxiety[55]. Leibowitz and Hollander has confirmed their work[56,57]. Since then, Sajdyk demonstrated that the infusion of lactate results in anxiety in rats. Significant changes in regional blood flow in panicking patients but not in the non-panicking patients occurs after lactate infusion[58]. The amygdala processes and directs inputs and outputs that are key to fear behavior. It directly senses that reduced pH and increased CO_2 content, inducing fear. Buffering pH attenuated fear behavior[59]. Anxiolytic drugs are, to a large extent, effective in ameliorating anxiety symptoms. Diazepam boosts mitochondrial respiration in the nucleus accumbens[60].This change of pH may be a consequence of change in the transport mechanisms of bicarbonate ions[61]. Likewise, several antidepressants with anxiolytic capacity have been reported to improve mitochondrial activity such as monoamine oxidase inhibitors[62], selective serotonin reuptake inhibitors (SSRIs)[63].

Table 2. Drugs decreasing entropy production.

Caloric restriction[41]
Anesthetics[43-45]
Sedative[46,47]
Anxiolytics[48]
Antiepileptic drugs[48-50]

C) Drugs increasing the release of entropy in the form of biomass

Metabolic syndrome is the consequence of diets rich in fructose. Intake of fructose causes an anabolic syndrome with increase in visceral adipose deposition and de novo lipogenesis[64]. The risk of developing cardiovascular disease and type 2 diabetes increases with the occurrence of metabolic syndrome. In the U.S., about 25% of the adult population has metabolic syndrome, a proportion increasing with age, particularly among racial minorities[65].

Insulin is an anabolic hormone. In type-1 diabetes, there is weight loss and hyperglycemia, which may result in coma. To the opposite, treatment with excess insulin may cause weight gain[66-68]. Long-term uses of corticosteroids have been used to increase muscle strength and performance. Anabolic steroids have been used to enhance recovery after massive stress and exhaustion[69].

Estrogen suppression results in anabolism. Ovariectomized rats eat more and gain weight more rapidly than sham-operated rats. Estradiol (E_2) treatment attenuates food intake and body weight gain in ovariectomized in rats[70]. Women gain weight at menopause.

Table 3. Drugs increasing the release of entropy in the form of biomass.

Diet rich in sugar[64]
Insulin[65]
Long-term corticosteroids [67-69]
Estrogen[70]

D) Drugs excreting entropy as waste products

From a thermodynamic standpoint, urine, and feces are waste products. Their elimination relieves the body from entropy-rich products. Diuretics, emetics, and laxative should be considered as lowering the entropy of the body. Radiation therapy (RT) is a therapy using ionizing radiation to control or kill inflammatory and cancer cells. RT has been extensively used for the treatment of inflammation, but this indication is slowly disappearing because of the risk of radiation-induced malignancies. RT may be curative in several types of cancer if they are localized to one limited area of the body. RT kills both cancer and normal cells. Cytotoxic chemotherapy activates the concentration of radicals species, such as the ones induced by radiation therapy. This is evident by the elevation of lipid peroxidation products; the reduction in plasma levels of antioxidants such as vitamin E, vitamin C, and β-carotene; and the marked reduction of tissue glutathione levels that occurs during chemotherapy. Those agents that generate high levels of Reactive Oxygen Species (ROS) include the anthracyclines (e.g., Doxorubycin, Epirubicin, and Daunorubicin), alkylating agents, platinum complexes (e.g., Cisplatin, Carboplatin, and Oxaliplatin), epipodophyllotoxins (e.g., Etoposide and Teniposide), and the Camptothecins (e.g., Topotecan and Irinotecan)[71]. After successful radiation therapy or chemotherapy, there is a sharp decline in the number of cancer cells, resulting in decreased tumor mass.

Table 4. Drugs excreting entropy as waste products.

Diuretics
Laxatives
Emetics
High dose cytotoxic chemotherapy[71]
Radiation therapy[71]
Surgery (organ removal)

E) Drugs increasing the release of entropy in the form of heat

Sport increases the activity of the mitochondria, thus the release of entropy in the form of heat. Increased activity has been shown to improve survival from cancer[72] and memory in Alzheimer's disease[73].

Inflammation is part of the complex biological response of body tissues to harmful stimuli, such as pathogens, or irritants and is a protective response involving immune cells, blood vessels, and molecular mediators. The function of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair. Inflammation (a clinical feature) is closely related to hyperosmolarity (a physical feature)[74-76]. Animal models of inflammation demonstrate that, in an inflammatory fluid, whatever its cause, there is an increased protein content resulting in increased osmolarity (oncotic pressure). On the other hand, increased osmolarity, whatever its cause, results

in inflammation[74-76]. Increased extracellular osmolarity increases cytokine synthesis and secretion and results in the proliferation and activation of immune cells. There is an inflammatory component in every major disease[4]. There is a concomitant rewiring of the metabolic fluxes, with an increase in secretion of lactic acid.

The increased pressure such as seen in inflammation inhibits the mitochondria and induces the secretion of lactic acid[77]. The increased secretion of lactic acid, a stigma of the metabolic shift toward anabolism, feeds on the inflammatory cells and plays a part in the immune response such as seen in all these diseases[4]. This is in line with the concomitant finding of inflammation, mitochondrial impairment, and lactic acid secretion in most chronic diseases. Intraperitoneal injections in rats of hypertonic solutions result in the secretion of lactate by the brain cells.

Non-steroidal anti-inflammatory drugs (NSAIDs) alleviate inflammation, the cyclooxygenase (COX) enzyme. COX synthesizes prostaglandins. NSAID decreases the synthesis of pro-inflammatory molecules by enhancing the mitochondrial activity[78].

There is also increased osmotic pressure in cancer[4]. Increased pressure has recently been discovered as one of the reason for the Warburg's effect[77] The Warburg's effect is present in all tumors[25]. To compensate the reduced energy yield, there is massive glucose uptake, aerobic glycolysis, with an up-regulation of the PPP resulting in increased biosynthesis leading to increased cell division. The massive extrusion of lactic acid contributes to the extracellular acidity and the activation of the immune system [4,24].

Anticancer drugs have been designed to kill cancer cells, but most drugs also target the Warburg effect, thus decreasing the synthesis of biomass and stimulating the excretion of entropy in the form of heat. Injection of radio labeled glucose (PET scan) allows assessment of the efficacy of treatment. The decrease in uptake of glucose correlates with the efficacy of radiation therapy[79], chemotherapy and hormonotherapy[80].

Accordingly, cancer treatment should aim at restoring the oxidative phosphorylation. Lipoic acid targets the pyruvate dehydrogenase and increases the oxidative phosphorylation[81]. Hydroxycitrate inhibits the citrate lyase and decreases the efflux of citrate into the cytoplasm, enhancing the energy yield of the mitochondria[82]. The combination of these two drugs decreases the growth of tumor and prolongs the life of the mice[82]. Inhibition of cancer growth appears universal (independent of the primary site).

Methylene Blue (MB) is an FDA drug discovered in 1878. It has already been rigorously studied and used in humans for over 120 years[83]. Methylene blue functions as an alternative electron carrier, which accepts electrons from NADH and transfers them to cytochrome c[84-86]. It decreases aerobic glycolysis and enhances oxidative phosphorylation. Methylene blue reverses the Warburg's effect and inhibits proliferation of cancer cell[87].

Antidepressants target the mitochondria at multiple levels [83, 84, 88]. Bachman studied the effects of five antidepressants, two phenothiazines and one butyrophenone on respiratory functions of rat heart mitochondria[89]. All compounds increased oxygen consumption and caused uncoupling of oxidative phosphorylation.

Table 5. Drugs increasing the export of entropy in the form of heat.

Sport[72,73]
Anti-inflammatory[78]
Low dose cytotoxic chemotherapy
Hormonotherapy
Drugs targeting the mitochondria/metabolism[85]
Antidepressants[88-89]
Drugs enhancing the memory[84]

CONCLUSION

In conclusion, we have been able to propose a qualitative classification of drugs for a wide range of diseases. Such classification derives directly from equation (1) that is based on the facts that living cells are open systems that are not at thermodynamic equilibrium. Rather, living cells, through their metabolism, produce continuously a large amount of entropy. Such entropy is released in part as heat, i.e. as infrared radiations having a wavelength larger than 10 μm (far-IR). In our classification, drugs do not change the entropy content of substances fueling their metabolism, which that are the three main ways of producing entropy. It should be clear that thermodynamics of irreversible processes allows derivation of equation (1). See references[23-25] for technical details. This explains why me must put focus on entropy rather than energy. The next step will be to associate to each drug its irreversibility potential (IrP). This would then allow moving from a qualitative classification to a quantitative one. If the corresponding computations are not difficult, there are tedious owing to the huge amount of drugs available in medicine.

It also follows from the presented approach that drugs can be repurposed. To convince the reader, we will take some examples. A first one is methylene blue (MB). This drug has a wide spectrum of action: malaria,

leprosy, depression, neurodegenerative diseases or more recently cancer[69]. In psychiatry, methylene blue has been used for over a century. It was tried successfully to treat psychotic and mood disorders and as a memory enhancer in fear-extinction training. Particularly promising results have been obtained in both short- and long-term treatment of the bipolar disorder. In these studies, methylene blue produced an antidepressant and anxiolytic effect without the risk of a switch into mania. Long-term use of methylene blue in bipolar disorder led to a better stabilization and a reduction in residual symptoms of the illness[83]. In addition to protect neurons, MB's effects have been associated with improvement of memory and behavior in a network-specific and practice-dependent fashion. Specifically, low-dose MB has shown cognitive-enhancing effects in a considerable number of learning and memory paradigms, including inhibitory avoidance, spatial memory, fear extinction, object recognition, open-field habituation and discrimination learning[83]. Yet another example is lithium that appears both effective in bipolar and in cancer[90]. Finally, it is worth noting that anticancer agents have been repurposed in the treatment of autoimmune diseases[91]. We do hope that the classification proposed here, based on physics and not on biology, will helps in repurposing old drugs. This would strongly reduce the cost of the treatments, with the additional advantage of escaping from troubles linked to patents. Accordingly, most old drugs are now in the public domain and would then be easily available for emergent countries.

Seen from a biologist's perspective, most metabolic pathways appear to be connected to each other. But from a physicist standpoint, they all point towards an increase in entropy flux within the body. Whatever the causes (i.e., genetic defect, inflammation, or toxicity of xenobiotics), they all converge toward a shift in the type of entropy that is released in the environment. In other words, most, if not all diseases have in common a decreased activity of the mitochondria. The synthesis of thermal IR photons decreases, and there is a concomitant increase in biomass synthesis. This can be addressed by the treatment of the primary cause (for example a genetic defect) or by medication targeting the mitochondria, such as Methylene Blue. To proceed toward a better outcome, the treatment needs to be evaluated and integrated into more comprehensive and global theories, accounting with principles of physics. It then follows that the goal of modern pharmacology may be to address treatment able to modulate entropy input or output to the desired organ.

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Historical Articles

History of Research on Phospholipid Metabolism and Applications to the Detection, Diagnosis, and Treatment of Cancer

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Abstract. In the past 30 years there has been a significant increase in the number of publications on phospholipid (PL) metabolism, both for the medical purposes of detection and diagnosis of cancer and for the monitoring of the treatment of human cancers. Most of the work has focused on the pathway that produces phosphatidylcholine, the major component of human cell membranes. The trigger for this research was the advent of applications of NMR spectroscopy *in vitro* and *in vivo* in the 1980's and observations that most cancer cells and tumors had significant increases in the water-soluble PL precursors and breakdown products. Increased phosphocholine (PC) has been focused on as a marker for cancer using Magnetic Resonance Spectroscopy (MRS) and Positron Emission Tomography (PET). MRS is now used clinically to aid in the diagnosis and severity of some brain tumors; and choline PET is used for the diagnosis and staging of recurrent prostate cancer, paid for by medical insurance companies. Another major area of research starting in the 1990's was the development of specific choline kinase (CK) inhibitors aimed at the isoenzyme CK-a. This isoenzyme is markedly upregulated in cancer cells and unexpectedly was found to have a role in oncogenic transformation independent of its enzyme function.

Keywords: phospholipid metabolism, phosphocholine, MRS, PET, choline kinase, cancer diagnosis.

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* List of Abbreviations used: **^{18}FCH,** ^{18}F-fluorocholine; **Ala**, Alanine; **BCR**, Biochemical Recurrence; **CK**, choline kinase; **CSI,** Chemical Shift Imaging; **CT**, Computed tomography; **DWI**, Diffusion Weighted Imaging; **FDA**, United States Food and Drug Administration; **FDG**, ^{18}F-fluorodeoxyglucose; **Ga-68,** Gallium-68; **GPC**, Glycerophosphocholine; **GPE**, Glycerophosphoethanolamine; **HC-3**, Hemicholinium-3; **HGG**, High grade glioma; **Lac**, Lactate; **mpMRI**, multiparametric MRI; **MRI**, Magnetic resonance imaging; **MRS**, Magnetic resonance spectroscopy; **MRSI**, Magnetic resonance spectroscopic imaging; **NAA**, N-acetyl-aspartate; **NMR**, Nuclear Magnetic Resonance; **PC**, phosphocholine; **PCr**, phosphocreatine; **PDE**, phosphodiester; **PE**, phosphoethanolamine; **PET**, Positron emission tomography; **PL**, phospholipid; **PME**, phosphomonoester; **PSA**, Prostate specific antigen; **PSMA**, prostate specific membrane antigen; **PtdCho**, phosphatidylcholine; **PtdEth**, phosphatidylethanolamine; **tCho**, total choline peak; **tCr**, total creatine peak.

INTRODUCTION

Lecithin was one of the first organic substances described, isolated by the French chemist Theodore Gobley from egg yolk in 1845.[1] The chemical structure of phosphatidylcholine, one of its main components, was not established until 1874.[2] Over the next century much work was done that led to our understanding of the metabolism of the PL components that make up the cell membrane of mammalian cells.[3]

At first PL metabolism in cells and tissues were studied by invasive techniques, such as cell lysis and extracts[4] and freeze-trapping.[5] However, it was eventually realized that these techniques gave unreliable results because of the rapid release of kinases that degraded the substances of interest. It was realized that noninvasive techniques were needed to quantitatively assess the levels of PL metabolites in intact cells and tissues.[6] Foremost among these methods was the use of noninvasive NMR spectroscopy (MRS) to detect phosphate-containing metabolites such as ATP using ^{31}P MRS as first observed by Mildred Cohn in 1960.[7]

Metabolism of intact cells was investigated by ^{31}P MRS using a perfusion technique with cells trapped in a gel[8, 9] and tissues were investigated *in vivo* using specially developed surface detection coils.[10] This included direct investigation of phosphate-containing metabolites in tumors grown on nude mice.[11, 12] These studies resulted in the observation that the levels of PL metabolites such as PC and phosphoethanolamine (PE) are higher in rapidly dividing cells such as cancer cells that are non-contact inhibited than in normal contact-inhibited cells.[13-15] Several authors have identified these studies as the trigger initiating interest in use of these findings in cancer diagnosis and detection.[16-20]

Parallel noninvasive studies were carried out using proton (^{1}H) MRS, but these were more difficult due to the presence of the huge H_2O solvent peak, requiring water-suppression methods.[21] For tissues *in vivo*, because of the greater sensitivity of the method, spatial localization techniques were developed using gradient methodology.[22, 23] Although these MRS methods demonstrated the basic observation that increased cell membrane biosynthesis could be used as a monitor of cancer cells, ^{31}P MRS was too insensitive and initially ^{1}H MRS was too cumbersome to be applied *in vivo* and in the clinic for human applications. A much more sensitive tomographic method was needed and that has become positron emission tomography (PET) that has allowed these research observations to be applied clinically to the detection and diagnosis of cancer.[24, 25] Eventually ^{1}H Magnetic Resonance Spectroscopic Imaging (MRSI) was developed to be more sensitive and less cumbersome and is now used clinically in brain tumors.[26]

Also, as a result of the differences between PL metabolism in cancer and normal cells, it was realized that kinase inhibitors could be effective anti-cancer drugs and this has resulted in the development of potential anti-cancer therapeutics.[27, 28]

PHOSPHOLIPID (PL) METABOLIC PATHWAYS

The two biochemical pathways for the two main components of the PL membrane in humans, PtdCho (phosphatidylcholine) and phosphatidylethanolamine (PtdEth), were worked out by Eugene Patrick Kennedy in 1956[29] and are commonly referred to as the Kennedy pathways (Figure 1).

Research on these two pathways has continued at a steady pace since 1956, but greatly increased starting in the late 1980s due to observations made using NMR Spectroscopy which was being used *in vitro* in cell suspensions and *in vivo* in animal and human tumors. These studies indicated these pathways were more active in cancer cells. The PL membrane makes up 70% of the dry weight of human cells and PtdCho and PtdEth make up to 70% of the lipid portion of the membrane.

The Kennedy Pathways,[31] are relatively simple three step pathways that are completely analogous. Furthermore, choline is trimethylethanolamine and the 3 extra

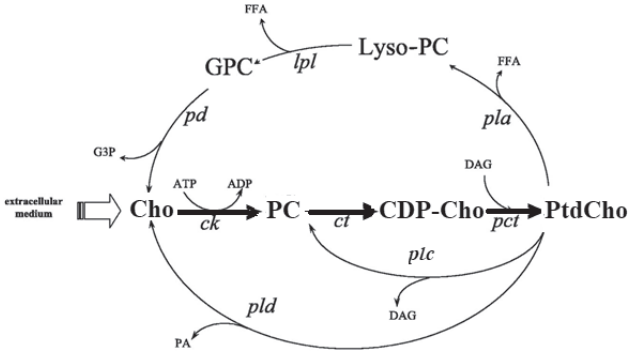

Figure 1. Kennedy pathway (center bold pathway) showing biosynthesis of the main PtdCho component of mammalian cell membranes from PC and the break-down pathway to glycerophosphocholine (GPC). A parallel pathway exists for PtdEth.[30] The enzymes involved in the pathways are shown in italics. Abbreviations: **CDP-Cho**, Cytidine diphosphate-choline; **Cho**, choline; ***ck***, Choline Kinase; ***ct***, Cytidylyltransferase; **DAG**, Diacylglycerol; **FFA**, Free fatty acid; **G3P**, Glyerol-3-phosphate; **GPC**, Glycerophosphocholine; ***lpl***, Lysophospholipase; **Lyso-PC**, Lysophosphocholine; **PA**, Phosphatidic acid; ***pct***, Phosphocholine transferase; ***pd***, Phosphodiesterase; ***pla***, Phospholipase A; ***plc***, Phospholipase C; ***pld***, Phospholipase D.

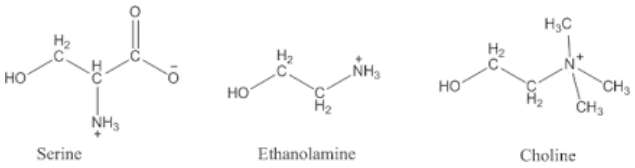

Figure 2. Chemical structures of ethanolamine and choline.

CH3 groups in choline (Figure 2) allow for it to be more easily observed in ^{1}H NMR spectroscopy *in vivo* and *in vitro* since the signal derives from the 9 equivalent H atoms of the trimethylated nitrogen atom.

Most of the research since 1990 has focused on the choline pathways. For the sake of simplicity, we will only show figures of the choline pathways although the ethanolamine pathways are completely analogous.[29]

The simple three step Kennedy pathway[32] for synthesizing phosphatidylcholine is:

Choline to Phosphocholine to CDP-Choline to Phosphatidylcholine.

This is the central synthetic pathway in Figure 1 going from left to right. The degradative pathways occur via the phospholipase A (*pla*), phospholipase C (*plc*), and phospholipase D (*pld*) pathways that are shown above and below the central synthetic pathway in Figure 1 going from right to left.

The metabolites that are seen in ^{31}P MR spectra of *in vitro* cell suspensions and *in vivo* are **PC** in the synthetic phosphatidylcholine pathway; and **PE** in the analogous synthetic PtdEth pathway (not shown) and **GPC** (Glycerophosphocholine) and **GPE** (Glycerophosphoethanolamine) in the degradative pathway starting with phospholipase A (*pla*). The degradative pathway via Phospholipase C (*plc*) contributes a small percentage of the **PC** peak in the NMR spectra.

The degradative pathways have also drawn interest not only because they produce **GPC** and **GPE** which are observed in the ^{31}P NMR spectra of tumors. But the degradative pathways also produce the metabolites phosphatidic acid (PA) via phospholipase D (*pld*); and diacylglycerol (DAG) via phospholipase C (plc) which are second messengers within the cell involved in multiple functions including growth and the mitogenic activity of growth factors via the RAS family of proteins.[33-36] The RAS proteins have enzymatic activity and exist in an "on" and "off" state. When turned on they trigger a cascade that ultimately turns on genes involved in cell growth, differentiation, and survival. Overactive signaling inside the cell can ultimately lead to cancer.[37]

CK is the first enzyme in the Kennedy pathway and has been found to be overproduced in almost all cancers beyond their need for phosphatidylcholine synthesis and has been under intense study in the past 25 years as a key enzyme in cancer and necessary for oncogenic transformation. CK also interacts with the RAS protein family for signal transduction and high concentrations of CK have been noted to turn on RAS proteins for signal transduction.[17, 38] Multiple CK inhibitors have been synthesized as potential chemotherapy agents for cancer.[27] As the first step in the synthetic pathway CK phosphorylates choline to **PC.** The next enzyme in the pathway is cytidylyltransferase (*ct*) which is rate limiting and PC accumulates and is easily seen in ^{31}P MR spectra *in vitro* in cancer cells and *in vivo* in tumors.

APPLICATION OF MRS

In 1980 Jack Cohen joined the National Cancer Institute with the intention of using NMR spectroscopy as a tool to study the metabolism of cancer cells. A Varian 400 MHz NMR spectrometer was purchased, and studies began in 1981. The basis of this work were the attempts made to devise a system whereby this noninvasive NMR technique could be used to study cancer cells *in vitro*. Previous attempts using suspensions of cells had proved unsuccessful, since the large number of cells (ca. 10^{9} cells) in 1 ml in a 10 mm ^{31}P MRS tube required to obtain sufficient signal-to-noise, used up all the available nutrients and became ischemic before any useful results could be obtained.[39]

Our first attempt to overcome this problem was to suspend cancer cells in an agarose gel and attempt to perfuse it with a solution containing nutrients and oxygen.[40] But this was not really successful. In order to enable the cells to metabolize, the solution had to be in contact with all of the cells as much as possible. We then devised a method to place the cell suspension in a liquid gel and flow it through a fine capillary (0.5 mm id) that was dipped in a container of ice, whereupon the mixture gelled and the cells were trapped and the spaghetti-like gel threads were then extruded into an NMR tube and could be perfused with the nutrient-containing and oxygenated solution and remain metabolically active for days[8] (Figures 3-5).

Using this technique we were able to see a high level of ATP in the cells as well as other metabolite signals (Figure 6), and by adding other metabolites or drugs to the solution being pumped through the cells we could monitor changes in the metabolism of the cells.[9, 42]

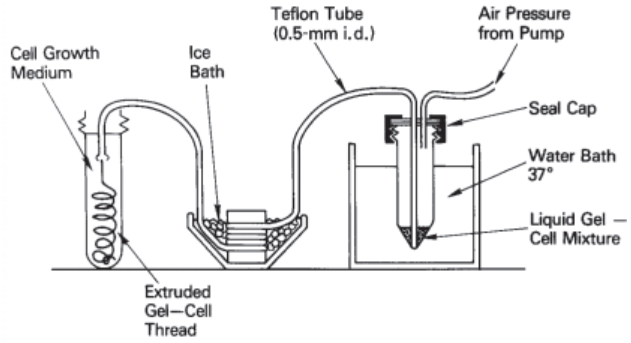

Figure 3. Diagram of the apparatus used to embed cells within agarose gel threads. A mixture of cells in medium is extruded through a fine Teflon capillary in chilled ice. The gel thread is then extruded directly into medium in the 10-mm screw-cap NMR tube.[41]

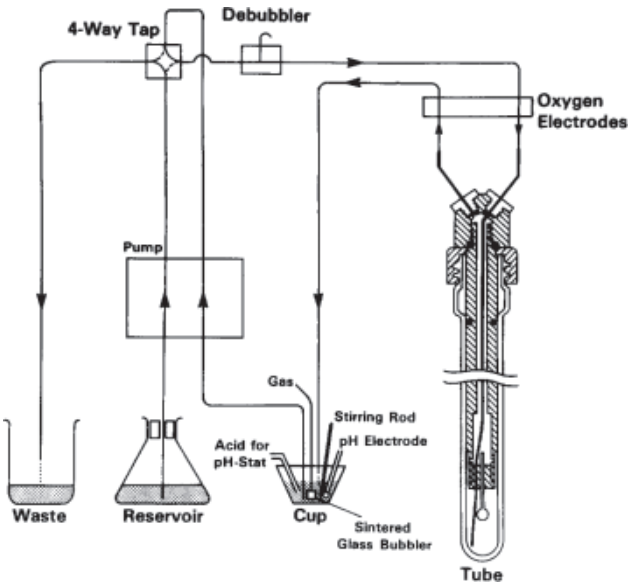

Figure 4. Schematic of the perfusion system showing the arrangement of the polyethylene insert.[41]

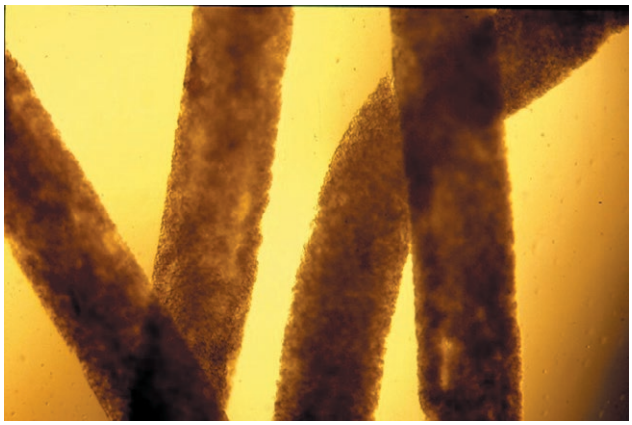

Figure 5. Photomicrograph of gel-threads showing cancer cells embedded in perfusable gel.[41]

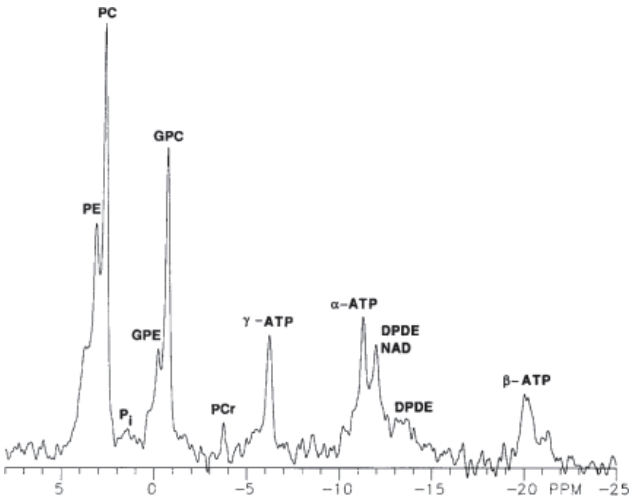

Figure 6. Representative ^{31}P NMR spectrum at 162 MHz of wild-type MCF-7 human breast cancer cells (~10^8/ml) perfused with IMEM media (Pi-free) in agarose gel thread (0.5 mm); 200 scans were accumulated with a recycle time of 40 sec and a 90° pulse. The peak assignments are denoted: PE, PC, Pi, inorganic phosphate; GPE, GPC, PCr, phosphocreatine; ATP, adenosine triphosphate, DPDE, diphosphodiesters; NAD, nicotine adenine dinucleotide.[41]

We carried out a series of studies that resulted in greater understanding of the metabolic response of several cancer cell lines grown in culture under different circumstances. [15,16, 43-46] In earlier studies the phosphomonoester (PME) peaks were erroneously assigned to sugar phosphates (SP), but we confirmed their assignment to PC and PE by the addition of choline and ethanolamine (separately) to the perfusion solution.[13] This was the first observation of the enzymes of the PL pathways functioning in *real time* in *intact cells* by MRS. On addition of ethanolamine, all four peaks, PC, PE, GPC, and GPE reacted to ethanolamine as expected by well-established substrate and inhibition effects. Ethanolamine inhibits CK and the phosphodiesterases that break down GPC and GPE to choline and ethanolamine, and it is the substrate for ethanolamine kinase producing PE. All four peaks can be seen reacting to the ethanolamine infusion as expected in Figure 7.[13]

One of our initial observations was that the peak assigned to PC and PE in the spectrum of cancer cells was found to be higher than expected (Figure 6) and higher than the same peak in *in vivo* studies of normal tissue that were used as controls.[47, 11] This important observation of elevated PC and PE in cancer cells was

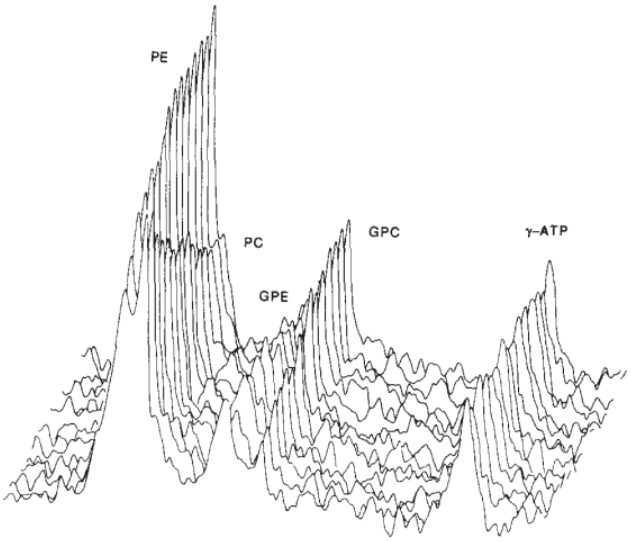

Figure 7. Effect of ethanolamine in the perfusate. Shown are quantitative ^{31}P NMR spectra of cells grown in IMEM medium with 15 mM choline and no ethanolamine, harvested at log phase, and then perfused with Buffer A, 11 mM glucose, plus 2 mM ethanolamine at 37°c. Each spectrum represents a l-hr accumulation. Hours 2 to 16 are shown.[13]

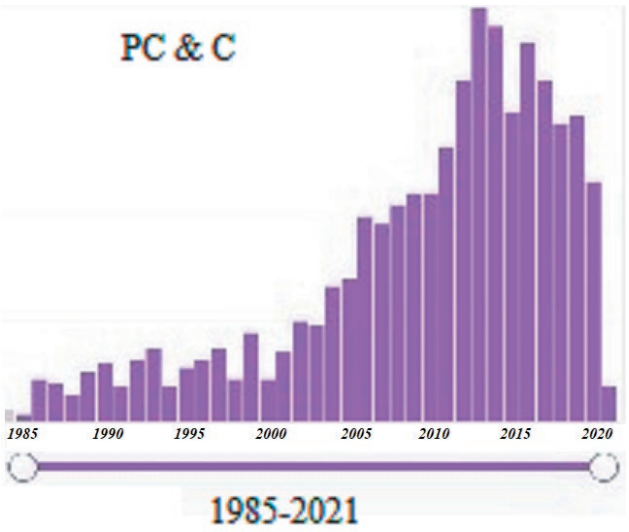

Figure 8. Plot of number of hits vs. year for a search of "phosphocholine and cancer" using the Scifinder (CAS) search engine, with 1,485 hits at maximum..

the basis for many future studies and has had significant influence in subsequent studies of cancer diagnosis and detection. In fact, in several reviews,[17-20] this observation has been singled out as the seminal observation that resulted in much greater research activity in this field (Figure 8). Note that a similar pattern of increase in research activity was found for every topic that was searched in this field.

This observation of increased PC in cancer cells was later confirmed by several authors using as controls noncancerous cells grown in culture.[38, 48] This subject has been extensively reviewed by Glunde and coworkers.[49] in which they document increased PC/PE in 6 different cancer types, including breast,[50, 51] ovarian,[52] prostate,[53, 54] cervical,[55, 52] brain[56, 57] and endometrial[58] cancers. Of significance was the observation of the PME to phosphodiester (PDE) ratio, the more malignant the cell line the more PC and PE were present and the less GPE and GPC were observed. It is this ratio that is more significant, not the absolute concentrations. In the first observation of this type in 1986, comparing the perfused wild-type cell line to the Adriamycin-resistant cell line derived from it, adding up the PME and the PDE concentrations, the wild-type had a PME/PDE ratio of ca. 2, but the resistant more malignant cell line ratio was about 16.[47] Some biochemists looking at choline metabolites call this the PC/GPC "switch" as the malignancy progresses.[38, 30, 19]

We later improved the perfusion technique by using basement gel membrane as the gel substance.[59] Meanwhile others had developed other methods of monitoring the ^{31}P MRS of cells using both suspensions aerated by oxygen[60] and bioreactors.[61, 62] It should also be mentioned that similar perfusion studies were performed with ^{13}C labelled metabolites observed by ^{13}C MRS[63] or by ^{1}H MRS using the ^{13}C-^{1}H spin-spin (J) coupling to gain higher sensitivity.[64, 65] Direct proton MRS studies of choline levels have also been measured.[66]

It should be pointed out that the signals of the PLs themselves are not observed in these ^{31}P spectra of cells, because they are extensively broadened by spin-spin (T2) relaxation due to their macromolecular structure and restricted motion leading to efficient T2 relaxation. By contrast, the metabolites that are smaller molecules with extensive molecular motion, even though within the viscous milieu of the cell, provide narrower resonances. In effect the cellular metabolites provide a ^{31}P MR spectrum that is in fact superimposed on the top of a very broad PL baseline.

DEVELOPMENT OF CHOLINE-PET SCANNING AS A DIAGNOSTIC METHOD FOR PROSTATE CANCER

The observations of the 1980s and their confirmation by further cell studies in the 1990s that the majority of cancer cells had unusually high levels of PC and GPC increased interest in using this fact as a way to diagnose and stage cancer; and to monitor and adjust cancer thera-

py. Since the 1990s the main problem with using MRS for these purposes was the low signal to noise ratio in MRS requiring large "voxels" or cubes of tissue for obtaining a good spectrum. With phosphorus spectra this required voxels that were multiple centimeters in diameter and would sample tissue other than the tumor, including normal tissue and necrotic tissue. ^{1}H spectra using proton MRS was developed and could obtain spectra from 1 cm^{3}. [67, 68, 23] While this was helpful with brain tumors it was still too large a volume for many other common cancers.

To overcome the signal to noise problem ^{11}C-choline for human Positron Emission Tomography (PET) scans was synthesized in 1997 by a Japanese Group led by Hara.[69] Historically, the first synthesis of ^{11}C-choline for PET scans was in 1983 and was used to observe normal brain tissue in a monkey.[70] The Japanese group however used their own synthesis as there were no details for the synthesis given in the 1983 paper. Radiolabeled choline has been used since 1997 in PET in cancer research for imaging brain and other tumors.[69, 71, 72] This is based on the first step in PL synthesis, choline being rapidly metabolized to PC by CK (Figure 1). In addition most cancers have increased membrane transport of choline compared to normal cells.[17]

The two forms of choline most commonly used in PET scanning are ^{11}C-choline and ^{18}F-fluoromethylcholine,[73] which is commonly called ^{18}F-fluorocholine (^{18}FCH). In ^{11}C-choline one of the ^{12}C carbons in the N-trimethyl group of choline (see Figure 2) is replaced by an ^{11}C atom. In ^{18}FCH, one the of the hydrogens in the N-trimethyl group of choline is replaced by ^{18}F. ^{18}FCH was first synthesized by DeGrado in 2000.[74, 75] They found that ^{11}C-choline and ^{18}FCH behaved similarly in cell cultures and also in their ability to be metabolized by CK. Later studies showed that ^{18}FCH was comparable in diagnostic ability to ^{11}C-choline, but is easier to use because of its longer half-life.[76-78] ^{11}C has a half-life of 20 minutes and ^{18}F of 110 minutes allowing for ^{18}FCH PET images to be obtained for a longer time, from 5 min to 60 min after injection. Other choline analogs were synthesized,[74] but these contained additional carbon atoms and were not transported as well by choline membrane transport proteins or metabolized as well by CK.[74]

Since CK is overexpressed in most cancers and rapidly metabolizes choline to PC; most cancers contain higher concentrations of PC compared to normal cells.[79] This generates a visible signal in the PET scanner. From the time ^{11}C-choline and ^{18}FCH were synthesized in 1997 and 2000 it was found that multiple tumors could be found by choline-PET, including brain, head and neck, breast, lung, esophageal, liver, kidney colorectal, prostate, bladder, uterine, ovarian cancers, and lymphomas.[80] For medical use though, it must be shown that it is better than other imaging methods, cost effective, and also has the ability to obtain an image in a time that is comfortable or tolerable for the patient. When PET is combined with a CT or MRI scanner it gives a more accurate location of tumors or metastases.[73] Since 2000 when the PET/CT was first invented most studies have been done with the PET/CT scanner.

The initial studies done from 1997 into the early 2000s showed that ^{11}C-choline or ^{18}FCH did produce clearly delineated images of tumors with a good signal to noise ratio. But for most tumors it was not better than ^{18}F-fluorodeoxyglucose (FDG) PET scans for tumors, which was already in widespread use. FDG works well because it is a glucose analog, and most cancers have a high rate of glucose uptake and utilization. And for brain tumors amino acid PET tracers were also superior.[73] However prostate cancer is an exception in that it is more slowly growing and does not absorb FDG rapidly. In addition, prostate cancer is one of the most common cancers in men with a high mortality rate. It was shown in 2003 that ^{18}FCH PET scans were superior to FDG for restaging prostate cancer after recurrence and this resulted in a marked decrease in the use of FDG for imaging prostate cancer and an increase in choline-PET,[73, 81] both for the initial staging and restaging of prostate cancer after relapse. Unfortunately, in prostate cancer relapse is high so comparative imaging in initial stage and relapse is of utmost importance. Prior to choline-PET tracers, staging was done using CT, MRI images, and ultrasound.

Most of the research on the clinical applications and actual clinical use of choline-PET has occurred in Europe, Japan, and Australia.[24] This is due to the difficulty of getting approval from the FDA (United States Food and Drug Administration) for new PET tracers and financial barriers such as uncertain reimbursement in the USA.[24, 82] ^{11}C-choline was approved by the FDA in 2012 but ^{18}F-choline has not been approved as of 2021, even though it was developed and tested in the USA at Duke University in 2000.[74] After initial studies from 1998 to 2003 showing the feasibility of choline-PET and choline-PET/CT there have been thousands of studies since 2003 focusing on the clinical applications of choline PET. ***The area that it has proven the most useful is in the restaging of relapsed prostate cancer.***

The use of choline PET/CT has become common since about 2010 in many parts of the world for the initial staging and restaging of prostate cancer.[24, 73, 83] A 2021 paper from France started by saying *"F-choline PET/CT is considered a cornerstone in the staging and restaging of patients with prostate cancer."*[84] Another

study published in 2020 focused on the "real world" use of choline-PET showed that it was commonly used for both initial staging and restaging and resulted in a change of therapy in 58% of the patients.[85]

In addition, new PET radiotracers have been developed in the past decade that focus on prostate specific membrane antigen (PSMA) or amino acid tracers such as ^{18}F-FACBC (Fluciclovine) for imaging prostate cancer; and studies are ongoing comparing the effectiveness of each of these tracers compared to ^{11}C-choline or ^{18}FCH.[24] Also, when using choline-PET in patients with prostate cancer, tumors other than prostate cancer are picked up incidentally in 1 to 2 % of patients.[84]

STAGING OF PROSTATE CANCER WITH CHOLINE-PET COMPARED TO MRI

Initial (Primary) Staging; Locating the cancer in the preoperative prostate

Numerous studies were done from 2003 onward evaluating the use of choline-PET scanning for the initial staging of prostate cancer.[73] One study from 2006 focusing on the local detection of prostate cancer nodules by ^{11}C-choline within the prostate preoperatively compared with biopsies done preoperatively[86] did show choline-PET could find 83% of prostate nodules that were 5 mm or greater in diameter, but only 4% of nodules smaller than that for an overall sensitivity of 66% compared to the biopsies. 5 mm is generally considered the lower limit in size detection for PET scans in general -- not just for choline studies.

Another study in 2010[87] looked at the preoperative evaluation of cancer nodules within the prostate as compared to preoperative biopsies, or examination of the prostate by a pathologist after surgical removal. A combination of standard MRI images combined with gadolinium enhanced images of the preoperative prostate found 88% of the nodules, ^{11}C-choline-PET found 73% of the nodules, and FDG-PET found only 31%. Multiparametric MRI (mpMRI) studies in 2015 using DWI (Diffusion Weighted Imaging) showed mpMRI to be superior for detecting cancer in the preoperative prostate as well as local extensions outside the prostate.[88] For modern prostate cancer imaging mpMRI uses four sequences: T1-weighted images, T2-weighted images, DWI, and dynamic contrast enhanced (DCE) imaging. Most commonly T2-weighted images with DWI and DCE are use or T2 weighted images with just DWI. In the past MRS of the prostate was also used as a fifth option to be part of the mpMRI workup but MRS of the prostate is not commonly used currently.

Lymph node and bone metastases

The two other areas of importance in the initial staging are metastases to nearby lymph nodes and bone. Choline-PET/CT was found in many studies to be superior to CT and conventional MRI scans at finding metastases to the lymph node. One reason may be that CT and conventional MRI rely mostly on the size and appearance of the lymph nodes whereas choline PET/CT that has a functional component was able to detect micrometastases to the lymph nodes.[89] The sensitivity of these studies was only about 50% however when compared to the lymph nodes that were removed at the time of surgery and examined by a pathologist.[89, 73] For this reason, The European Association or Urology in 2021[90] still recommends lymph node removal for proper staging of prostate cancer at the time of initial diagnosis. Since PET/CT images the entire body, one important value of choline-PET/CT is that it can detect lymph nodes with prostate cancer outside the area of a standard MRI scan or outside the surgical field of a standard lymph node dissection.[91-93] For these reasons choline-PET/CT for detecting metastases to lymph nodes has been commonly used in Europe since 2010 (Figure 9).[73]

For staging of bone metastases choline PET/CT has consistently shown more accuracy than bone scan in its ability to detect both bone and bone marrow metastases (Figure 10).[94] It also has higher image resolution.[95-97] In patients with intermediate to high-risk prostate cancer it was found that choline-PET/CT was more sensitive and specific at detecting bone marrow metastases than bone scan or CT alone. For bone metastases they reported a 100% sensitivity and a 90% specificity with choline-PET compared to bone scan.[95] One study showed an advantage of choline-PET/CT over MRI or MRI DWI for detecting bone metastases in 47 high risk patients.[88] It should be noted that many of the studies were performed on intermediate to high risk patients. In the

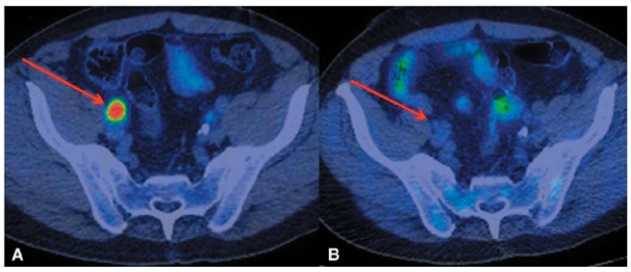

Figure 9. A shows a right external iliac lymph node with a large uptake of ^{11}C-choline. **B** shows the same area after 4 months of successful treatment.[24]

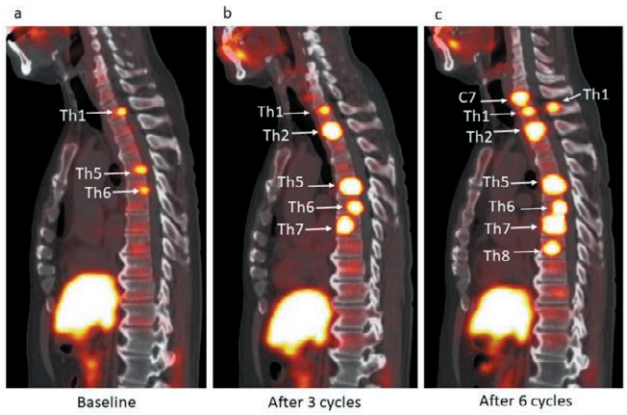

Figure 10. a to **c** clearly shows the progression of bone metastases during multiple cycles of treatment. The large bright spot in the lowest part of the figure is the normal liver which concentrates the ^{11}C-choline that is injected. Th is thoracic vertebrae, C is cervical vertebrae.[94]

higher risk patients, the sensitivity of choline-PET/CT increases dramatically.

Restaging after Relapse (Treatment Failure)

Many men treated for prostate cancer relapse and need to be restaged. In the vast majority of cases the relapse is found by an increase in the Prostate Specific Antigen (PSA). This is also called biochemical recurrence (BCR) in prostate cancer. PSA is an enzyme found predominantly in the cytosol of prostate cells.[24] For most men without prostate cancer the normal serum level is about 0.7 ng/ml and in prostate cancer it can increase dramatically to 100 or 1000 ng/ml but in most cases levels above 6 to 10 are worrisome. Most men treated for high-risk prostate cancer have the prostate and pelvic lymph nodes removed at the time of initial diagnosis. In these men the PSA level goes below 0.2 ng/ml. An increase of the PSA on two measurements taken 3 months apart indicates recurrence. For men treated only with radiation therapy without removal of the prostate an increase of 2 ng/ml from the lowest measurement during treatment indicates recurrence, sometimes called biochemical failure.[73]

As in the initial staging, mpMRI proved to be superior to choline-PET scanning for locating a recurrence in the prostate or in the prostate area of men who had undergone prostatectomy.[98, 99] Where choline PET/CT stood out was its ability in restaging after treatment failure to detect lymph node and bone metastases.[83] Choline PET/CT showed an overall sensitivity for recurrence in the lymph nodes, bone, and other sites in 86 to 89% of patients and its use is recommended by the European Association of Urology.[90, 100-102] This far exceeded the detection rate of FDG-PET and mpMRI. For detection of all lymph nodes compared to the surgical dissection of the lymph nodes after recurrence, the sensitivity was about 60%.[103-107] These sensitivities make it far superior to FDG-PET[81, 105] and mpMRI[99] for restaging of local and more distant lymph node metastases.

Of note, Choline-PET/CT findings at restaging have allowed for site directed radiation therapy to target the area and to calculate the radiation dose. This directed "salvage radiation therapy" by choline-PET/CT has led to improved disease free survival.[108-110] One study showed that a combination of salvage lymph node dissection and radiation therapy in patients led to a 70% five year disease free survival in patients staged with choline-PET/CT.[111]

In addition to its usefulness in detecting relapse into the lymph nodes choline-PET/CT was able to detect prostate cancer in 15% of patients with negative bone scans and is equivalent to mpMRI for detecting bone metastases.[101, 99] Another advantage of choline-PET/CT is its ability to detect the more aggressive osteolytic metastases.[112] This allows corrective treatment to be started to prevent fractures of those bones. For these reasons, choline-PET/CT is often the preferred imaging technique in relapsed prostate cancer treatment failures[24, 73, 83] and is commonly recommended in treatment guidelines.[113, 90]

Historical Guidelines for use of Choline-PET for Prostate Cancer

The European Association of Urology (EAU) Guidelines on Prostate Cancer[90] first mentions the use of choline-PET/CT in the 2010 guidelines for locating metastases to bone during initial staging, but states that the use in relapse is unclear. By 2015, the guidelines did not recommend choline-PET/CT for initial staging. But for staging in relapse, choline-PET/CT was useful for detecting lymph node and bone metastases if the serum PSA level was greater than 1 to 2 ng/ml and was more sensitive than bone scan. By 2018, the EAU guidelines gave a ***strong*** recommendation for the use of choline-PET/CT or PSMA-PET/CT following BCR after radiotherapy to stage local lymph node metastases or distant metastases to bone or other tissue. The 2021 guidelines discuss that choline-PET/CT can be used for detecting bone (but not lymph node) metastases at initial staging and will simultaneously detect bone and other more distant metastases, but it is not a strong recommendation. For cases involving relapse after radiotherapy the 2021 EAU does give a strong recommendation for choline-PET/CT or

PSMA-PET/CT for patients being considered for curative lymph node salvage treatment and can change management in up to 48% of patients.

It is noticeable that the formal recommendations are more conservative than what is reported above as actual common use of choline-PET/CT in initial staging and restaging. However, the recommendations are consistent with publications highlighting that it is more sensitive in cases of relapse than at the initial staging. One reason for this may be that the biochemical studies on cancer cells show that the levels of PC increase as the cells become more malignant. With relapse it is the more malignant and aggressive cells that predominate.

PSMA PET scan for prostate cancer

PSMA is a folate hydrolase glycoprotein[24] that, despite its name, is found in more tissue than just prostate. Despite the similarity of the name this is a different protein enzyme than Prostate Specific Antigen (PSA) which is mostly in the cytosol of prostate cells. In prostate cancer PSMA is overexpressed by 100 to 1000-fold. A newer PSMA PET agent Gallium-68 PSMA-11 was recently approved by the FDA in Dec 2020[114, 115] and has been used and studied in Europe and Australia since 2015.[116, 117] Many recent papers claim it is more sensitive and specific than choline-PET at lower levels of PSA and future head to head comparisons are being planned. There have been other PSMA-PET tracers developed. Since PSMA is an enzyme, they have in common that they target the substrate recognition site on PSMA. To date it is Gallium-68 PSMA that has been the most studied. It should be noted that "PSMA PET tracer" refers to the whole family of PSMA tracers and not to any one in particular.

Correlation with Prostate Surface Antigen (PSA) level for Choline and Gallium 68 (Ga-68)

It has been noticed that there is a strong correlation between the sensitivity of a PET imaging agent to detect prostate cancer in patients, and the serum level of PSA. This is because the higher the serum PSA the more advanced the cancer. It has become the norm to divide the sensitivity of PET imaging agents into 3 categories. The sensitivities below a PSA of 1 ng/ml, those sensitivities for PSA levels between 1.0 to 2.0 ng/ml and the sensitivities for a PSA level above 2.0 ng/ml.[24] A recent review[24] in patients with a relapse showed Ga-68 PSMA to be more sensitive at all levels. The authors cautioned that this was pooled data from multiple studies, and they were not standardized. However prospective head-to-head studies of choline vs Ga-68 PET/CT scanning is underway.

Multiple studies published in 2020 and 2021[116, 118, 117] also show that Ga-68-PET/CT is more sensitive than choline PET/CT and is replacing choline PET/CT in many centers. However, the experience gained with choline PET/CT over the past 20 years cleared the way and is influencing and guiding the applications of Ga-68 PET/CT. Basically, they are the same applications such as staging and guiding of therapy but with a more sensitive agent. It remains to be seen if choline-PET/CT will still have applications that Ga-68 cannot be used for in prostate cancer and other cancers.

CHOLINE KINASE AS A TARGET OF CHEMOTHERAPY

The earliest PL enzyme target for chemotherapy and the one most studied has been CK.[119] However other enzymes in the pathway have also been proposed as targets.[120] The development of CK inhibitors for possible cancer treatment has been closely connected to the biochemical studies on this enzyme and its importance in two different functions: 1) cancer transformation and 2) the catalyzing of choline and ATP to produce PC. By 1998, it had become clear that there were two forms of CK – an alpha and a beta form and their amino acid sequence had been determined.[121] Subsequent studies showed CK alpha to be the more important isoform in cancer.[122] To date the most potent CK alpha inhibitors developed have been MN58b and RSM-932A which is also called TCD-717.[27, 119]

The first paper published on interfering with the PL pathways by CK inhibition was in 1974.[123] The compound used was purinyl-6-histamine and had been observed to be cytotoxic to tumor cells *in vitro* with little to no effect on normal cells *in vitro* or to have any effect on DNA or proteins. The study made "preliminary observations" about purinyl-6-histamine's effects as a CK inhibitor and the morphological changes observed in the membrane of cancer cells being more pronounced than those of normal cells, but there were no follow-up publications.

There were two more papers published in 1983[124] and 1985[125] studying the effects of Hemicholinium-3 (HC-3) on Krebs II ascites carcinoma cells. They found that HC-3 inhibited both the choline transport mechanism across the cell membrane and also inhibited CK intracellularly. They also found that the synthesis of PtdCho was diminished in Krebs II ascites carcinoma cells by HC-3 but not in normal liver cells.

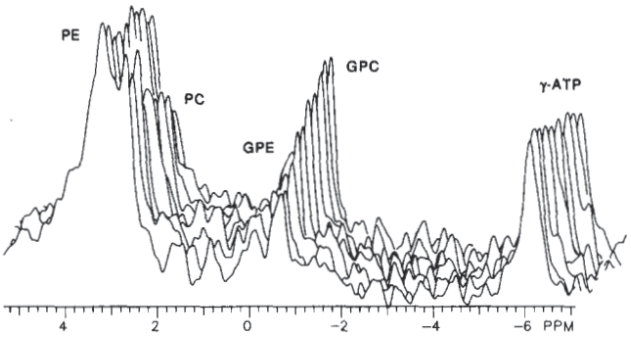

Figure 11. Effect of HC-3 in the perfusate. Quantitative 31P NMR spectra are depicted of cells grown in IMEM with 15 mM choline and 10 mM ethanolamine, harvested at log phase, and then perfused with Buffer A, 11 mM glucose, plus 100 mM HC-3. Each spectrum represents a 1-h accumulation.[13]

In 1987 the ^{31}P NMR spectra of MDA-MB-231 cancer cells were monitored while being observed in intact cells being perfused in the NMR spectrometer by a buffer solution without any choline. The introduction of HC-3 into the perfusate caused a reduction of the PC peak by over 50% in 8 hours (Figure 11).[13] This would indicate that the reduction in the PC peak observed in this experiment was due to inhibition of CK and not only to inhibition of choline transporters. HC-3 would become the template that most future CK inhibitors would be based on.[27, 119]

As discussed in the section on "Applications of MRS," subsequent studies done in the 1990's confirmed that most cancer cells studied had high levels of CK and PC. A review article by Podo referred to 1983 to 1993 as the "pioneering decade"[19]. MRS studies on cells grown *in vitro* led to the hypotheses that the increased PE and PC levels in cancer cells were involved in cell membrane synthesis, and cell growth in cancer cells,[13, 126, 127, 15] and that "specific oncogenes resulted in the increased production of choline and ethanolamine kinase."[16] These observations triggered further studies and confirmation of the hypotheses involving the PL pathways and the enzymes and oncogenes involved.

Most of the studies have focused on CK.[17, 120] Further studies showed that the CK alpha gene also functions as an oncogene involved in tumor initiation and progression.[120, 128, 129] Of the alpha and beta forms only CK alpha has been found linked to tumor transformation. Increased expression of CK alpha 1 is oncogenic to cells, but overexpression of CK beta is not. Increased production of CK alpha 1 mRNA is found in breast and lung cancer cell lines, but there is no change in CK beta mRNA levels.[122]

From 1987 to 1995 multiple papers were published showing that the ras oncogene causes an activation of the CK enzyme[130, 34, 131-136] causing an increase in PC. Data from one of the papers[34] indicated that PC may function as a second messenger in cells involved in cell growth. At that time HC-3, which was first reported in 1974[137] was the most potent inhibitor of CK and served as the template for the development of numerous CK inhibitors that fall into two categories: 1) bis-pyridiniums and 2) bis-quinoliniums.[27, 119] HC-3 itself had a paralyzing respiratory effect at therapeutic levels for treating cancer,[138] so CK inhibitors were developed that were both more effective with no or reduced side effects *in vivo*.[27, 119]

In 1997, numerous bis-pyridiniums were produced in the lab of Juan Carlos Lacal[131] and the compound named MN58b proved to be the most effective. NIH3T3 cell lines that had been transformed by ras, src, and mos oncogenes were profoundly inhibited in their growth by the new bis-pyridiniums CK inhibitors by a factor of 600 to 1000 and were effective in the low micromolar range.[139] The ras, src, and mos transformed cells had elevated levels of CK activity and the degree of inhibition correlated with reduced production of PC. MN58b was later shown to significantly inhibit the growth of xenograft tumors.[140, 139, 141] Later studies showed that MN58b was 20 fold more effective at inhibiting the CK alpha enzyme compared to the beta form of the enzyme.[27, 122]

The next set of new CK inhibitors studied were in the bis-quinolinium class. In 2005 Lacal's laboratory studied forty more compounds and compound 40 was the most potent in the bis-quinolinium class.[142] It was subsequently labeled as RSM-932A and is also called TCD-717. TCD-717 completed a Phase 1 study in solid tumors in 28 patients between Jan 2011 and February 2014.[143] TCD-717 was selected for the Phase 1 trial as it has no detectable toxicity in mice at levels that cause 77 percent tumor growth inhibition *in vivo*. In addition, it caused reversible cell cycle arrest in nontumor cells but cell death via apoptosis in cancer cells.[144] The study was sponsored by Translational Cancer Drugs Pharma which is located in Spain, but the studies were conducted at Johns Hopkins University and the Barbara Ann Karmanos Cancer Institute in Detroit, Michigan. To date results of the Phase 1 trial have not been published, but in 2021[119] Lacal referring to the Phase 1 trial stated that "the toxicology studies have already been addressed" and indicated that RSM932A/TCD717 has "paved the way for future development".

RSM932A/TCD717 has a unique mechanism of action and was found to ***not*** bind to the pockets on CK alpha where choline and phosphate are catalyzed like

other CK alpha inhibitors bind, but to bind to the surface of the enzyme.[145] This caused both a severe reduction in both PC and in the levels of CK alpha. One hypothesis is that RSM932A/TCD717 causes a drastic reduction of the level of CK alpha protein by causing a conformational change that makes it susceptible to proteases.[119] In some tumors inhibition of CK alpha with no reduction in CK alpha levels within the cell is insufficient to cause cell death.[146, 147] However, in glioblastoma cells inhibition of CK alpha by pure enzyme function inhibitors such as V11-0711 that has no effect on the level of the enzyme is sufficient to lead to tumor cell death.[119] The mechanism of cell death in tumors by CK inhibitors may vary depending on the cell type.

A 2021 review article titled "Recent Advances in the design of small molecule CK alpha inhibitors and the molecular basis of their inhibition" shows that this remains an active area of research.[28] In addition to small molecule inhibitors, siRNAs (silencing RNAs) have also been developed against CK alpha and phospholipase D and are under active study and produce reduced cell proliferation in xenographic tumors but have not yet reached Phase 1 patient studies.[120]

DEVELOPMENT OF CLINICAL APPLICATIONS OF MRS/MRSI/MRI

Introduction: Magnetic Resonance Spectroscopy (MRS) of tissue metabolites in a freshly amputated frog leg was first reported in 1974 by D.I Hoult et. al. in George Radda's research group at Oxford where they observed phosphate metabolites by ^{31}P MRS.[148] In that first report they labeled the observed peaks as sugar phosphates, PL, Pi, creatine phosphate, and the gamma, alpha, and beta peaks of ATP. This was one year after the first paper on the feasibility of Magnetic Resonance Imaging (MRI) was published by Paul Lauterbur.[149] Since the late 1980s MRI and MRS have been closely linked, with localized MRS being recorded after an MRI is obtained.

In 1980 the earliest *in vivo* human MRS was reported by J.D. Cresshull et. al. in Radda's lab observing the ^{31}P spectra from a human arm and the effects on PCr (phosphocreatine) and Pi before, during, and after the removal of a tourniquet.[150] The first *in vivo* spectrum of a human tumor was done by ^{31}P MRS in 1983 by Griffiths et al.[151] of a rhabdomyosarcoma and showed prominent peaks in the PME region and the PDE region not seen in normal muscle. The PME area is now known to be PE and PC but was assumed to be sugar phosphates at that time.

In 1985 *in vivo* ^{31}P MR spectra of neuroblastomas in two children with metastases to liver and muscle showed a high concentration of PMEs in the tumor compared to the ^{31}P NMR spectra of the normal liver and muscle tissue *in vivo* in the same children, which showed much smaller PME peaks. The large PME peak returned to normal size if the neuroblastoma was successfully treated or if it spontaneously regressed (a common feature for neuroblastoma in infancy). This initial observation of 1) a high PME peak that 2) diminishes with resolution of the tumor has been one of the main uses of MRS in cancer ever since. The PME peak in neuroblastoma was assigned to PE at 10 mM concentration, with a smaller contribution from PC and 2,3-DPG (2,3- Disphosphoglycerate).[152] They also reported a small PDE peak which in high resolution ^{31}P MRS of biopsy material was identified as GPE and GPC.

As was discussed earlier in the section on Application of MRS, extracts and cell studies were also done concurrently with the early *in vivo* ^{31}P MR studies. At that time there were conflicting reports of the origins of the PME peak in cells and *in vivo* from tumors. Many early ^{31}P MR studies assigned the PME peak to sugar phosphates. Others assigned the PME peaks to PC and PE using acid extracts of cancer cells,[19] but the enzymes creating these peaks were not determined. In 1987 the first ^{31}P MRS studies of cancer cells in real time *in vitro* studied with enzyme substrates and inhibitors (Figure 7) confirmed the PME peaks were predominantly PE and PC; and they were produced by CK and ethanolamine kinase in the Kennedy pathways, and the PDE peaks were mostly GPC and GPE.[13]

By 1989 *in vivo* ^{1}H spectra of human brain and brain tumors were being published which showed multiple metabolites in the ^{1}H spectra, including a large peak that was a combined peak of choline, PC, and GPC called the total choline peak or tCho peak.[153, 23] Biochemical studies established that the dominant peak in the tCho peak is PC with a contribution from GPC and a smaller contribution from free choline.[17] Since that time most *in vivo* spectra of cancer in humans have been ^{1}H spectra since it has greater sensitivity and can be obtained from smaller volumes than ^{31}P spectra.[18] Because MRS can measure some metabolites localized *in vivo* it has always held out the promise that it could be useful for diagnosis and monitoring of treatment.

It is the unique radiofrequency of the two hydrogen atoms in water that provide the images obtained by clinical MRI machines. The hydrogens in fat also contribute somewhat to the images. Water is 55.5 Molar which means the two hydrogen atoms in water are at 111 Molar. Due to this high concentration the ^{1}H radi-

ofrequency of water observed from the human body produces a very strong peak with an extremely high signal to noise ratio. Because water does not make up 100% of the volume of human tissue the signal is correspondingly decreased. By contrast, the concentration of other metabolites seen by ^{31}P or ^{1}H MRS such as PC, PE, GPC, GPE are in the range of 1 to 10 millimolar or .001 Molar to .01 Molar. This is a concentration difference of approximately 10,000 to 100,000 fold and makes obtaining spectra from patients reliably, quickly, reproducibly, easily, and with a high signal to noise very difficult and has been the major barrier to the widespread use of MRS in medicine, despite major attempts over the past 30 years.[154, 155] As of today, common use of single voxel MRS or multivoxel MRSI occurs in major research hospitals, but not in community hospitals where the vast majority of MRI machines are located.

The two main MR nuclei used *in vivo* have been ^{31}P and ^{1}H spectra. Of the two, ^{1}H has been used more often since ^{1}H has 16 times intrinsically more signal intensity than ^{31}P.[148] Also PC and GPC have 9 hydrogens in the trimethyl part of choline that give off the identical radiofrequency signal (see Figure 2) that also increases the signal to noise ratio. The main disadvantage of ^{1}H spectra is that choline, PC, and GPC have their radiofrequency signal so close together that *in vivo* it is just one peak called the tCho peak.

Although *in vivo* spectra have been used in areas other than cancer, these studies mostly involve peaks in the spectra not related to PL metabolism.[156] Whereas its use in aiding in the diagnosis of cancer and monitoring of therapy has relied predominantly on the PC and GPC peaks; and to a lesser extent, the PE and GPE peaks when ^{31}P NMR is used. The three areas of cancer where it has been used the most is in brain, breast, and prostate cancer. As of 2021, 354 clinical trials were found under the search "cancer and magnetic resonance spectroscopy" at clinicaltrials.gov. About 80% of NIH clinical trials have been in brain, prostate, and breast cancer, in that order.[157]

MAGNETIC RESONANCE SPECTROSCOPY AND SPECTROSCOPIC IMAGING IN BRAIN TUMORS

Localized ^{1}H MRS of the human brain was first reported in 1985[158] and multiple papers on high resolution ^{1}H MRS of the human brain soon followed.[159, 160] As of today multiple resonances can be observed by ^{1}H MRS of the brain and brain tumors which include tCho, NAA (N-acetyl aspartate), total Creatine (tCr), Glutamate/glutamine (often abbreviated Glx), Lactate (Lac), Alanine (Ala), Lipids, Myo-inositol, and a broad macromolecule peak.[161, 26] Usually the tCho peak is just called "the choline peak" and the tCr peak is just referred to as "creatine". 2-Hydroxyglutarate was first seen in 2012.[162] By 1989 Frahm et. al reported on 8 primary brain tumors and one metastatic breast cancer tumor to the brain.[153, 160] They found the spectra were remarkably different from normal brain tissue by having a high tCho peak and a low NAA peak. But histologically similar tumors gave similar spectra to each other. Similar results were reported on spectra obtained at 4 Tesla in 1989.[163] The tCho peak has been found to be predominantly PC[164, 26] and NAA is a marker of normal neuronal tissue.[165, 26] These papers looked at gliomas, meningiomas, one neurilemmoma, one arachnoid cyst, and one metastasis due to breast cancer. They concluded ^{1}H MRS may become an important tool for differentiation of tumors as well as for planning and following therapy. They also concluded that the ^{1}H MRS method was better than ^{31}P MRS since spectra could be obtained on smaller voxels which avoided sampling both the tumor and the surrounding normal tissue.[163]

These early studies were single voxel studies. However, in 1982 Truman Brown published on NMR chemical shift imaging (CSI)[166, 167] where spectra are obtained from multiple voxels adjacent to each other in a square grid of n by n voxels; and then a gray or color scaled image of the intensity of a metabolite such as choline can be made from the individual spectra in each voxel. This can be extended to a cube that is n by n by n voxels. If it's a flat grid it's 2 dimensional CSI and a cube is 3 dimensional CSI. CSI is now frequently referred to as MRSI (See Figure 13 from 2021). Because the metabolites are in such low concentration compared to water these images do not give the same high resolution as standard MRI but methods for increasing their resolution have improved markedly since the late 1980s.[168]

In 1990 Luyten et al. produced MRSI of brain tumors on voxel sizes of 1.225 ml (7 by 7 by 25 mm) and produced low resolution images showing elevated tCho and decreased NAA in tumors with noticeable heterogeneity within the same tumor.[161] By 1992 ^{1}H NMR spectra on over 200 brain tumors had been reported. Some of these reports used CSI but most used single voxel spectra.[169] As of 1992 the most common observations on primary brain tumors were an elevated tCho, decreased tCr and decreased NAA (See Figure 12 from 2003).[170] tCho, tCr, and NAA are the three most prominent peaks in the ^{1}H spectra of brain and brain tumors. Metastatic cancers to the brain and gliomas frequently contained Lac whereas meningiomas, neurinomas, and lymphomas did not. Meningiomas often contained Ala.

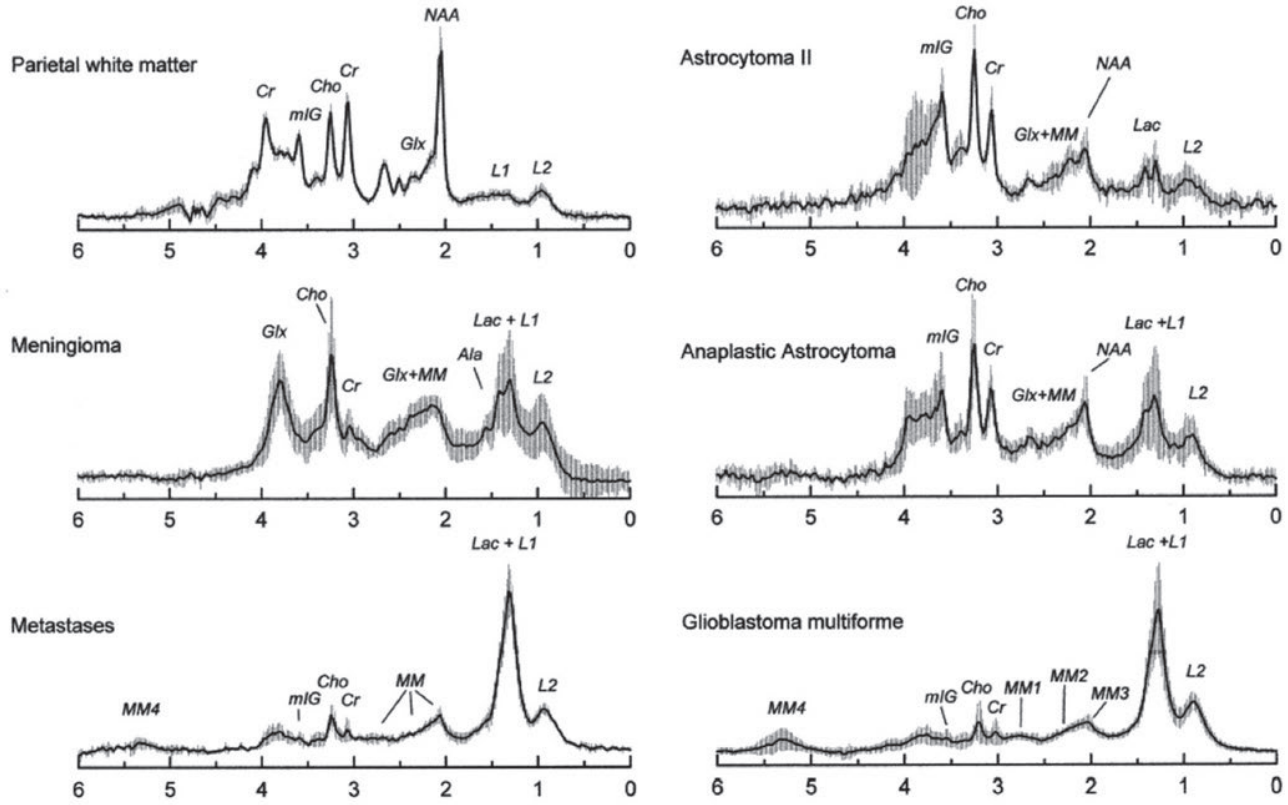

Figure 12. Spectra of normal brain tissue (parietal white matter) compared to tumors. The increased choline and decreased or absent NAA in the tumors is well demonstrated. Cr is tCr, mIG is myo-Inositol, Cho is tCho, Glx is glutamate plus glutamine, NAA is N-acetyl aspartate, L1 and L2 are lipids, Lac is lactate, MM is macromolecules, Ala is alanine which is characteristic of meningiomas.[170]

Increased PME and decreased PCr were not as commonly observed with ^{31}P MRS of brain tumors due to the large voxel sizes that would also include normal and/or necrotic tissue and would dilute the tumor signal.[169] By the mid to late 1990s most brain MRS was ^{1}H MRS. Increased tCho and decreased NAA and decreased tCr are still the most reliable change in the spectra from normal to malignant brain tissue seen in ^{1}H MRS or MRSI of brain tumors.[26]

In 1996 a pilot study using ^{1}H MRS was done to see if it could distinguish between recurrent or residual brain tumor vs delayed cerebral necrosis in children following radiation therapy since this could not be done by standard imaging techniques.[171] 12 children were studied by ^{1}H MRS and the results were confirmed by biopsy. Markedly decreased tCho, tCr, and NAA were expected to indicate necrosis and easily visible tCho and tCr was expected to identify residual or recurrent tumor tissue. Biopsies were done after the spectra and MRS identified 5 out of 7 patients with tumor and 4 out of 5 patients with necrosis. The conclusion was ^{1}H MRS showed promise for differentiating necrosis from tumor. As of 2021 this is one of the common clinical uses of ^{1}H MRS.[26]

In 1996 and 1998 Preul et al used pattern recognition analysis of the six most common peaks observed in ^{1}H NMR spectra in the 1990s.[172, 173] For each profile, the metabolites were plotted by connecting peak heights with a straight line in the order with which the metabolites appear in a ^{1}H-MR spectrum from left to right: tCho, tCr, NAA, Ala, Lac, and Lipids. The most common brain tumors are gliomas, so called because they derive from glial cells. Glial cells are not neurons but non-nerve cells that support the neurons of the brain. There are 4 types of glial cells: astroctyes, oligodendroctyes, ependymal cells, and microglia. The gliomas are graded I to IV in increasing grade of malignancy. Sometimes a tumor will be referred to as a Grade II glioma or sometimes more specifically as a Grade II Astrocytoma, or Grade II Ependymoma etc; if the glial cell of origin is identified by histology. An Anaplastic Astroctyoma is a grade III Astroctyoma. Glioblastoma multiforme is a Grade IV Astrocytoma. Grade I to II are low grade. Grade III to IV are high grade.

Preul et al. reported they could correctly classify 104 out of 105 spectra which included normal brain tissue and the five most common adult brain tumors that are shown in Figure 12. Biopsies of the tumors were done after the spectra were obtained. They concluded ^{1}H MRS can "enable accurate, noninvasive diagnosis of the most prevalent types of supratentorial brain tumors".[173] However, this paper did not include the spectra of other brain disorders such as abscesses, necrosis, lymphomas, and tumefactive demyelinating lesions. It was later found that brain tumor spectra can overlap with these pathologies and this overlap reduces the accuracy of ^{1}H MRS of brain tumors to 60 to 80% and has been the primary reason MRS is not used more frequently in the diagnosis of brain tumors.[26]

By the end of the 1990s into the early 2000s pattern recognition techniques were being used by several groups to diagnose different types of tumors by ^{1}H MRS or MRSI.[174-176] In Europe a multicenter project called "INTERPRET" was set up to give computer-based decision support to radiologists in diagnosing and grading brain tumors. Spectra were collected from 334 patients from 2000 to 2002 and used automated pattern recognition.[177, 178] Another project called eTUMOUR from 2004 to 2009 expanded the INTERPRET approach.[177] But these techniques have not been easy to transfer to general radiology clinical practice due to difficulty of obtaining spectra, the overlap in appearance of spectra of different tumors, and heterogeneity within the same tumor.[179]

CURRENT ESTABLISHED CLINICAL AREAS:

The studies done from 1989 to 2010 established the areas that are most useful now for ^{1}H MRS/MRSI in brain tumors. Further studies from 2010 to 2021 solidified these areas. Those areas are: **1) Diagnosis**, particularly of masses on the MRI that can mimic primary brain tumors in appearance, **2) Grading** of tumors which also relates to prognosis, **3) Post treatment evaluation**, especially for differentiating growth of the tumor from radiation effects, and **4) Treatment planning** for biopsy, surgical resection, and radiation therapy.[26, 180] The first 3 are used clinically and the fourth area is currently an active area of research.[26] The clinically useful areas are covered by some insurers.[180, 181] By 2020 it was established that **elevated tCho and reduced tCr and NAA** in primary brain tumors are the most important observations and the most useful ratios are tCho/NAA and tCho/tCr. The metabolites most used by 2021 are tCho, NAA, tCr, Lac, Lipids, and Myo-inositol.[26]

1) Diagnosis

The diagnosis of brain tumors up to 2010 has been previously discussed. From 2010 onwards more studies contributed. In the past the standard approach was first a needle biopsy of the tumor followed by surgical removal. On the MRI it may be difficult to tell a brain tumor from **metastatic disease, tumefactive demyelination, lymphoma, edema, abscess, or necrosis**. And spectra of high-grade gliomas (HGG) can overlap with other primary brain tumors and non-neoplastic disease, so MRS is not used alone but in combination with MRI and other imaging techniques This combined imaging can form a "virtual biopsy" on some, but not all, brain tumors before surgery to differentiate primary brain tumors from these other masses and the needle biopsy may not be needed.[26, 182]

One study on 69 adults from 2008 attempted to differentiate between tumors and their mimics by MRSI combined with perfusion imaging.[183] 36 of the 69 adults had brain tumors and the other 33 adults had a different diagnosis. The MRSI correctly classified 84% of the 69 lesions by using the ratios of NAA/tCho, NAA/tCr, and tCho and NAA normalized to signals from a normal area of the brain. However, when the MRSI findings were combined with perfusion imaging the specificity increased to 92% for the correct categorization. In another study from 2006 of 32 children the specificity was 78% for correct categorization, 13 of the children had tumors and 19 had a benign lesion.[164]

Both **metastases** and gliomas have elevated tCho and decreased NAA compared with adjacent normal tissue. But lipids and macromolecules can appear in the ^{1}H spectra and tend to be higher in metastases compared to gliomas.[184, 185] A study done in 2013 showed an 80% specificity using this method[26]. This illustrates that the additional peaks in the ^{1}H spectra of brain tumors add to the diagnosis rather than just relying on the increased tCho and decreased NAA levels. Gliomas often have microscopic extension into the surrounding brain tumor not seen on the MRI whereas metastases usually do not. Metastases tend to have a sharper border on the MRI. Studies published from 2004 to 2018 showed that spectra from the **edematous area** surrounding gliomas tend to have a high tCho/NAA and tCho/tCr.[184, 186, 187, 188] One study found this could discriminate a primary glioma from a metastasis with 100% sensitivity and 89% specificity.[189]

Primary central nervous system lymphomas tend to have a lower tCho/NAA ratio than primary brain tumors and lower myo-inositol.[190, 191, 192] **Tumefac-**

tive demyelinating lesions (TDL) overlap in appearance with primary brain tumors on MRI.[193] One report in 2018 found that a tCho/NAA ratio of greater than 1.72 was more consistent with HGGs than TDL.[194] Also TDL frequently has a high Lac peak usually not found in untreated brain tumors.[26] Spectra from **brain abscesses** were found to be fairly distinct. In 1995 and 2004 it was published that they tend to have decreased tCho, tCr, and NAA and often have signals from amino acids not seen in tumors.[195, 196] Similar results were found for abscesses in 2010 and 2014.[193, 197] Other observations made during 1989 to 2010 were that **necrotic areas** have low levels of most metabolites but an increased lipid signal.[179]

2) Grading of Tumors and Prognosis

Another area of importance is whether it is a high or low grade tumor. Low grade is grade I to II, high grade is III to IV. Where high grade are the more malignant tumors. Multiple studies found that the tCho level in astrocytomas correlated with the grade of the tumor. The higher the tCho level the more malignant the tumor.[164, 180, 168, 198] However, in 1993 and 2003 some high grade astrocytomas were found to have low levels of tCho perhaps due to the higher grade tumors having necrotic centers[179, 199] It was found in 2000 the spectrum could vary greatly depending on which part of the tumor was sampled.[200] ***By 2009 this led to MRSI being preferable since it accounts for the heterogeneity of tumors and necrotic areas and the voxel with the highest tCho signal can be chosen for spectral analysis and biopsy.***[201] One study in 2007 using perfusion imaging to correlate with the spectra found there was no difference in the spectra of high grade vs low grade gliomas *in areas of* ***low*** *blood perfusion*. But in the regions *with* ***high*** *blood perfusion,* the tCho, plus the glutamate plus glutamine peak; and Lac plus lipid peak, were higher in high grade vs low grade gliomas.[202] Low grade gliomas tend to have a modest choline elevation and a modest NAA reduction and usually do not have Lac peaks or lipid peaks. HGGs have more noticeable change from normal brain tissue including markedly increased tCho and decreased tCr, NAA, and myo-inositol.[203] Since decreased tCr is seen the tCho/tCr is usually higher in HGGs than in low grade gliomas. The presence of Lac and lipid peaks is more typical of Grade IV gliomas and not common in Grade III gliomas.[185] But there is still overlap in the appearance of the spectra of high vs low grade gliomas, so the use of ratios is helpful. A meta-analysis of 1228 cases in 2016 found that the tCho/tCr, tCho/NAA, and NAA/tCr ratios were the most helpful and had specificities in the 60 to 70% range.[204]

Numerous papers have been published up to this time on using MRS for prognosis (prediction of survival) independent of histologic grade.[205, 206, 207, 208] Most papers noted a high tCho/NAA ratio, and the presence of Lac and lipids were associated with a shorter survival rate in adults. A related finding was also made in pediatric brain tumors. In one paper on 76 children low tCho and low (Lac +lipid) levels compared to tCr were found to be a strong predictor of survival.[209] 2-HG (2-Hydroxyglutarate) has been used more frequently since it was first seen in 2012 and it's detection strongly indicates gliomas with isocitrate dehydrogenase mutations which tend to be low grade.[162, 26]

3) Post Treatment Evaluation

MRS has been found to be useful in differentiating between tumor progression or persistence versus radiation necrosis on the MRI following radiation treatment.[210, 211] In up to 24% of glioma patients receiving radiation therapy, radiation necrosis can develop.[179] Multiple publications from 1996 to 2017 showed increased tCho compared to normal brain tissue, or increased tCho/tCr ratios, or tCho/NAA ratios suggested recurrent tumor; whereas reduced tCho, NAA, and tCr levels implied radiation necrosis.[171, 211, 212, 213, 214, 215] In addition, radiation necrosis areas frequently showed increase lipid and Lac signals compared to tumors.[216, 217, 218] In a meta-analysis of 447 cases published in 2014 MRS has a specificity of differentiating tumor from radiation necrosis of 83% by using the tCho/tCr ratio.[219] Another meta-analysis of 203 patients published in 2017 showed MRS has performed better than other radiology techniques at differentiating radiation necrosis from tumor with a sensitivity of 91% and a specificity of 95%.[220]

4) Biopsy and Treatment Planning

^{1}H MRS or MRSI was suggested as early as 2006 and 2008 to be useful in guiding the biopsies of tumors and planning the therapy based on tumor extent and aggressiveness including targeting radiotherapy.[221, 222] This is currently an active area of research using whole brain MRSI.[26, 168, 223]

Zhong et al recently used tCho and tCho/NAA maps to guide surgical biopsies by targeting areas of high tCho or high tCho to NAA.[26, 168] (see Figure 13). Zhong et al also proposed MRSI maps such as these could be used in the future to plan radiation therapy, as have other studies.[224, 225] A future strategy would be to treat areas of highest tCho/NAA with higher dose radiation therapy. A

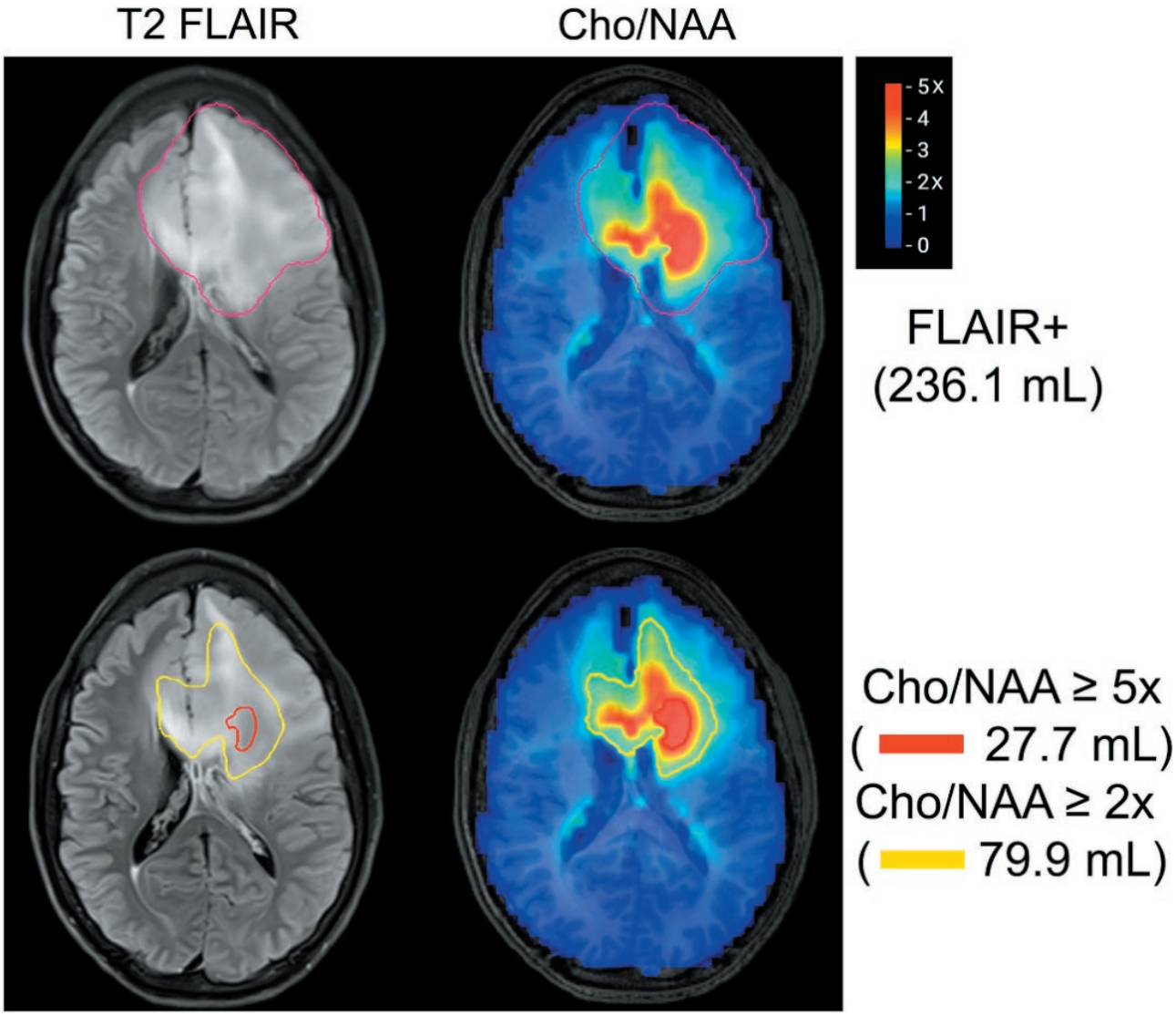

Figure 13. Upper Left Image: FLAIR image of a Grade II Astrocytoma with segmented volume of hyperintensity (pink outline) compared to Upper Right Image: T1 image overlaid with an MRSI colormap of tCho/NAA ratio. Lower Right Image: Outlined volumes show boundary for tCho/NAA of 2x (yellow) and 5x (red). The areas of highest tCho/NAA were targeted for biopsy. The upper and lower images are the same images, only the outlines are different. The tCho/NAA from normal contralateral white matter was set as equal to 1.0. Reprinted by permission from Springer Nature, Reference 168, Copyright 2021.

multisite trial was just completed and a phase II study is being planned.[226, 227]

CURRENT ESTABLISHED TECHNICAL ADVANCES IN MRS/MRSI

A major technical advance from 2010 to 2021 was a 10-fold improvement in spatial resolution. It was recognized in 2010 that one of the major limitations of MRS and MRSI was the large spatial resolution of 1 cm^3 for MRSI and 4 to 8 cm^3 for single voxel MRS was a limiting factor.[179] This greatly improved by 2021. The resolution in MRSI in Figure 13 is 0.1 ml but new developments may soon produce 2 mm by 2 mm by 3 mm resolution or 0.018 ml voxels.[168]

Currently it is mostly university research centers and a few clinical centers that are using MRS and MRSI for the clinical purposes discussed.[154, 228] The overwhelming need for transferring already existing technology at research centers for MRSI to current non-research clinical MRI machines for neuroimaging was addressed in a

2021 consensus statement written by multiple experts at leading research centers worldwide.[154] The authors pointed out that ***MRSI methods on clinical MRI machines have remained little changed in the past 20 years despite technical improvements over the past two decades that have greatly improved the quality of MRSI at research facilities producing the quality seen in Figure 13***. These improvements, primarily software updates, should bring brain MRS to the point of being an imaging modality (MRSI) and the review of the actual spectra (MRS) would be secondary. The authors pointed out they were recommending methods and uses that have already been demonstrated for neuroimaging that could be transferred to clinical practice at their current stage of development.

MRSI uses smaller voxels and can sample areas of the brain that single voxel spectroscopy cannot. The consensus group also reviewed the use of 7T MRI machines for spectroscopy although these machines are currently at research centers only and not at standard clinical radiology departments. The MRSI shown above (Figure 13) is from a 3T MRI.[168] While most clinical MRI scanners are 1.5T, commercial 3T scanners are becoming more common at both research and clinical MRI centers[229]. These higher field magnets greatly improve the speed of acquisition and resolution of MRS and MRSI. There are already many 7T MRI scanners at research centers and in 2017 clinical 7T MRI scanners were cleared for clinical use in both Europe and the USA.[230] These improvements should lead to further expansion of the reimbursement from more insurers for brain MRS and MRSI than currently exists.[180, 181]

PROSTATE AND BREAST CANCER MRS/MRSI

In addition to brain tumors, prostate and breast cancer are the other cancers that have received the most interest. But brain masses have had the most clinical success. This is due both to the nature of brain masses and technical facility. Multiple tumors can metastasize to the brain and on MRI there are multiple tumor mimics and MRSI combined with MRI is often the best method for diagnosis short of biopsy. Multiple metabolites can be seen, making MRS more useful; and both the skull and the highly sensitive nature of brain tissue make biopsy and surgery a much higher risk. Also, it is technically easier to do MRS/MRSI on the brain as it is a large organ compared to the prostate, the ability to keep the head still in a comfortable position with an MRS head coil and avoid motion artifacts due to breathing is a major advantage; and brain tumors tend to be fairly large at the time they are found.

The first ^{1}H spectra of prostate cancer using a transrectal probe was published in 1990 and showed a high citrate peak in normal prostate, and a low citrate peak in prostate cancer.[231] The first *in vivo* ^{31}P MRS of prostate cancer was published in 1991 and showed a high PME peak and PCr peak.[232] By 1996 three dimensional MRSI of the prostate with 0.24 to 0.7 ml voxels was done and multiple papers had been published showing a high tCho level and low citrate level in prostate cancer compared to normal prostate tissue.[233] The high tCho/Citrate ratio in prostate cancer is analogous to the high tCho/NAA ratio in brain tumors where tCho is a marker of malignancy and low citrate is a marker of lack of normal prostate cells. By 2012 the European Society of Urogenital Radiology (ESUR) was endorsing MRSI of prostate cancer as part of mpMRI studies of the prostate for diagnosis after relapse and for judging tumor aggressiveness and monitoring treatment response. However, they also pointed out that DWI did the same thing.[234] By 2020 mpMRI involving T1, T2, DWI, and DCE (dynamic contrast enhanced) imaging was in common use but MRSI was not.[235] Much of this had to do with technical difficulties including the use of an endorectal coil and the fact that DWI had turned out to be quicker, easier, more reproducible, and could give the same information for diagnosis, judging aggressiveness, and monitoring of therapy due to the highly cellular and unusually dense nature of prostate cancers that severely inhibits diffusion of water.[236, 155] However, further technical developments in MRS and MRSI such as avoiding an endorectal coil will likely make MRSI of the prostate useful and are still being studied.[234, 155]

By 1988 ^{1}H and ^{31}P MRS of human breast cancer *in vivo* were published. The ^{31}P spectra showed high levels of PMEs, PDEs, ATP, and Pi compared to normal tissue and these peaks reduced with successful treatment. The ^{1}H spectra showed a high water to fat ratio for the tumors of 2.2 but only 0.3 on average for normal breast tissue.[237] And by 1989 more publications appeared describing the use of ^{31}P NMR spectroscopy for monitoring breast cancer treatment *in vivo*.[238, 239] These papers noted both the increased PMEs in tumors and their decrease with treatment. By 1991 extracts of surgically removed tumors showed the broad PME region was predominantly PC and PE and the PDE region was mostly GPE and GPC as had been found in tumors in nude mice and cell studies.[16] Although many studies were done in the 1990s of breast tumors by ^{31}P MRS[240, 241] by the early 2000s most spectra were ^{1}H MRS, since it could be performed on a tumor one tenth the size and analysis of multiple ^{1}H MRS studies showed a sensitivity of 83% and a specificity of 85% at detecting breast

cancer based on the tCho peak. The specificity rose to 92% if ^{1}H MRS was combined with MRI.[242] Unlike brain and prostate there is not an additional metabolite in the breast ^{1}H MRS spectra as a marker for normal tissue similar to citrate or NAA so the determination is based almost solely on tCho. Also, there are technical problems caused by very large fat peaks in breast tissue not found in prostate and brain spectra.[156] A 2014 review pointed out that the development of MRS for breast cancer lagged behind the developments of MRS for brain and prostate but still felt it should be useful for diagnosis and monitoring of therapy.[243] But by 2019 the conclusion was still that MRS of breast tumors was "promising" and "proven to have a role in clinical care" but further work was needed in improving the technique.[244] To date MRS is not reimbursed by insurers for either prostate or breast cancer and is still considered experimental.[180]

FUTURE TRENDS IN PHOSPHOLIPID RESEARCH AND CANCER

One obvious area for future exploration is the role of the ethanolamine Kennedy pathway in cancer metabolism. Most tumors have large amounts of PE in them, often more than of PC, but this area has not been well studied biochemically or in MRS, and ethanolamine kinase-1 is found to be elevated in breast and prostate cancer cells.[245] In addition, the degradative pathways for both PtdCho and PtdEth have not been as well studied although these pathways are involved in some of the production of PC and presumably PE as well as producing second messengers involved in cell growth. And these enzymes may also be a target for therapy in addition to CK or in combination with CK inhibitors.[18, 49] And as discussed earlier, work continues on the development of CK inhibitors[119] and using MRSI of brain tumors for guiding biopsy and treatment and for prognosis.[26]

Another area that is developing is the use of ^{1}H MRS *ex vivo* on biopsy tissue or tumor tissue removed at surgery by High Resolution Magic Angle Spinning (HR-MAS). As the name implies HR-MAS involves placing tissue in a tube that is then spun at a specific angle calculated from NMR physics that results in improved signal to noise and higher resolution allowing many more metabolites to be seen than *in vivo* MRS.[246, 247] In addition, Chemical Exchange Saturation Transfer (CEST) and hyperpolarized ^{13}C are being studied.[49] Certainly, any future as yet undiscovered physical technique for in vivo MRS/MRSI that can greatly increase the signal to noise level will have a profound impact.

Choline PET is being explored for use in hepatocellular carcinomas[248] and in hyperparathyroidism.[249] Hepatocellular carcinoma is slow growing and, like prostate cancer, does not avidly take up FDG for PET scanning. Hyperparathyroidism often involves nodules of parathyroid tissue which can be difficult to localize but show up well on Choline PET scanning and the technique has been shown to be useful in a recent meta-analsis.[249]

While this is not intended to be a definitive list of all the future areas of research involving choline metabolism and choline MRS/MRSI, even a cursory review of the literature shows this to still be a very rich area for research.

CONCLUSION

The *sine qua non* of medical research is the ability to go from "bench to clinic," from basic research in the laboratory to the application to human healthcare. The subject that we have described the history of here, namely the study of PL metabolism, is an excellent example of that process. We have shown that from the earliest studies of PL metabolites in intact living cancer cells *in vitro*, it was found that the precursors and catabolites of PL can be indicators of the presence of cancer. These observations, primarily obtained by using ^{31}P MRS, were then extended and applied *in vivo* by both MRS and the vastly more sensitive methodologies of PET scanning and MRSI, to detect, diagnose and monitor treatment of patients in the clinic. This is a subject of growing clinical importance that has received the imprimatur of the medical profession and is now covered by healthcare organizations worldwide.

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Historical Articles

Capillary Electrophoresis (CE) and its Basic Principles in Historical Retrospect. Part 3. 1840s –1900ca. The First CE of Ions in 1861. Transference Numbers, Migration Velocity, Conductivity, Mobility

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Abstract. Since electrophoresis is a physical phenomenon – it is the movement of dispersed charged particles relative to a liquid under the influence of a spatially uniform electric field – its history is not limited to its use as a separation method. The history of capillary electrophoresis in particular, i.e. electrophoresis in capillary-sized open tubes, therefore does not begin in the 1960s, as is commonly assumed, but already a century earlier, if one refers to its principles. Capillary electrophoresis of ions was first performed by the French physicist Edmond Becquerel in 1861, about the same year as that of colloidal particles. Becquerel owns therefore the priority. It was subsequently performed on three other occasions in the *Long Nineteenth Century*, by Wilhelm Beetz in 1865, by Wilhelm Ostwald and Walther Nernst in 1889, and by Friedrich Kohlrausch and Adolf Heydweiller in 1895. All of these experiments were carried out in the context of research on conductivity and ion migration. Based on the theories of Grotthuß, Davy, and Faraday, it was believed until the 1840s that both the anions and the cations of a dissolved strong electrolyte – to which this review refers – migrate at the same velocity or speed in an electric field, but experimental observations in the mid-1840s cast doubt on this view. Wilhelm Hittorf was the first to show that these ions could migrate at different speeds, still consistent with Faraday´s laws. He was able to prove his hypothesis with experimental data and determined the migration velocities of the two types of ions in an electrolyte relative to the sum of their velocities, which he termed "Überführungszahlen" (transference or transport numbers). However, they did not initially yield the absolute velocities of the ions. This was achieved later by F. Kohlrausch, who devoted four decades of his research life, namely from the end of 1860 to about 1910, to the study of the conductivity of electrolyte solutions and the migration of ions. He discovered in 1879 that ions move independently from each other in solution (1st Kohlrausch law). It is remarkable that until the late 1880s it was generally believed that free ions do not exist in solutions in the absence of an external electrical force, but that ions were always tightly bound to their counterions. This belief dated back to Grotthuß in 1805. Although Rudolf Clausius hypothesized in 1857 that free ions are actually present in solutions as result of their thermal motion, this did not find further resonance. It is also remarkable that during this whole period under consideration no attempt was ever made to separate ions with the same charge, although their different migration properties were already known. Continuing his research,

Kohlrausch found empirically in 1900 that at extremely low concentrations the molar conductivity of ions, i.e. the conductivity related to their concentration, is a function of the square root of their concentration and approaches a certain limit at infinite dilution (2nd Kohlrausch law). As a precursor to this law, he derived in 1885 for larger concentration ranges the little-known relationship of molar conductivity as a function of the cubic root of concentration. He calculated the migration velocities of ions from their conductivities and characterized the migration behavior by their mobility, which is a central property in electrophoresis. Kohlrausch was certainly a formative investigator of the electrophoretic properties of ions, but his work focused mainly on strong electrolytes. This review covers the research results in the field of mainly this class of electrolytes in the period from 1840 to about 1910; but it also reports on the personal background of some researchers who, despite important contributions, have been unjustly forgotten, as well as on researchers who were active outside the scientific community. Mention is made, for example, of Gustav Theodor Fechner, who was the first to prove the fact, indispensable for electrophoresis, that Ohm´s law also applies to electrolyte solutions. However, in contrast to the generally applied results of his investigations, he himself was rather ignored by later researchers. The conductivities and electrophoretic properties of weak electrolytes, which were known to Kohlrausch and his contemporaries but hardly explicable to them, at least until 1884, are not discussed in detail in this review. In that year, Svante Arrhenius published his groundbreaking theory of electrolyte dissociation as his dissertation. This theory and the resulting consequences for the whole subject of electrolyte solutions require, however, a separate historical retrospect.

Keywords: first capillary electrophoresis, ions, strong electrolytes, Hittorf, Clausius, Kohlrausch.

INTRODUCTION

Although the motion of dissolved charged particles by electrophoresis[1] during electrolysis was the topic of Part 2 of this series[4] the magnitude of their migration velocities was not subject of discussion.[2] That part covered the period from the first observations of electrolysis and the inextricably linked electrophoresis in 1800, and the assumption that electrical forces from the electrodes exert on the ions by action at a distance. This concept was superseded in the mid-1830s by Michael Faraday's pioneering theory that ions move at electric lines of force, which led to James Clerk Maxwell's field theory. [5] At the time when the action at a distance was widely accepted, the migration velocity of the ions was believed to vary with their distance from the electrodes, while as consequence of the field theory the assumption of a constant velocity prevailed. In no case, however, was the magnitude of the velocity of ion migration addressed. This will be the subject of this Part 3.

In short, and just for the sake of historical completeness, we mention the first attempt to measure the migration velocity by Peter Mark Roget,[3] which he already undertook in 1807. Roget tried to determine the migration speeds of oxygen and hydrogen generated by the electrolysis of water,[4] but without success, as described in refs. [6, 7], Chapter *Galvanism*, p. 30. Michael Faraday then took up the subject only in 1833 in the *Fifth Series of Experimental Researches* (refs. [8, 9]; 524. – 535.). In the *Eighth Series*[10] from 1834 he explicitly argued that both the anions and the cations of an electrolyte travel at the same speed in opposite directions at the electric lines of force, and based this assumption on the law of the definite electrochemical action. He argued that equal chemical equivalents of oppositely charged ions are required at their respective electrodes at the same time, otherwise electrolysis cannot happen. We discussed this point already in Part 2.[4]

Faraday briefly returned to the subject of migration velocities of particles in solution in 1838 in the *Thirteenth Series*.[11] At the end of this article he reviewed earlier attempts to measure the speed of electricity in metallic conductors, which had been unsuccessfully carried out by William Watson in London in 1747 and 1748. [12, 13] That of light was carried out (with some success) by Charles Wheatstone in 1834.[14] Faraday himself speculated on an approximate velocity of the ions in solu-

[1] To avoid misunderstandings, we indicate how we define electrophoresis. It is not just the migration of particles in an electric field, which, like colloids, have an electric double layer. Nor is it just the powerful separation method in use today, whose overwhelming importance, for example, for genomics, proteomics, metabolomics, and numerous other important areas we highlighted in Part 1.[1] It is in principle, as Hanns Lyklema most generally defined it, "*the movement of dispersed particles relative to a fluid under the influence of a spatially uniform electric field*". [2][3] We therefore have every reason to call such migrations electrophoretic (see also Part 2 of this series).[4]

[2] It should be noted in advance that the present part of our historical review deals mainly with single pure strong electrolytes in free solutions.

[3] The British physician Peter Mark Roget (1779, London – 1869, West Malvern, Worcestershire) was Fullerian Professor of Physiology an der Royal Institution from 1834 till 1837.

[4] Roget tried to measure the time interval between the closing of the electric circuit and the appearance of the gases on the separate electrodes, which were 46 inches (i.e., ca. 117 cm) apart. After the completion of the circuit, however, no measurable interval could be determined; a result which was predicable taking into account the already known theories of Grotthuß and Davy.

tion, (ref. [11], pp. 164, 165) but only vaguely.[5] In none of his contributions, however, Faraday reported on values of the migration velocities.

The assumption of equal ion velocities was challenged by Frederic Daniell and William Allen Miller by contradictory results of the electrolysis of copper and of zinc sulfate solutions in a double membrane cell, i.e., a cell divided by two diaphragm. After electrolysis, they found the same amount of copper in the cathode compartment as was initially put in. The amount of reduced copper plus the quantity that remained dissolved was exactly that as before electrolysis. From this, they concluded that the copper ions do not migrate and only sulphate traverses the entire distance between the electrodes. They hypothesized that the flow of the electrophoretic current[6] was mainly by the anions, while the cations contributed little.[15, 16]

Claude Servais Pouillet observed a similar effect in 1845 when he electrolyzed a solution of gold chloride.[7] He used a U-shaped tube with one of the electrodes in each arm. After passing the current, he found almost no gold in the arm with the negative electrode, but gold in its initial content in the one with the positive electrode. Pouillet concluded from this result that the negative electrode took up the gold, while the chloride was transported to the positive electrode through a series of decompositions and recombinations and was set free there. Like Daniell and Miller, Pouillet concluded that the migration velocities of the anions and the cations are not the same, contrary to previous belief.[19, 20] Alfred Smee[8] had a different point of view. He assumed that the primary process is the decomposition of water when a metallic solution (i.e., a solution of a salt of a metal) is subjected to galvanic action. The following reduction is a secondary process caused by the hydrogen produced first by the metal from its salt solutions.[21, 22] In summary, all experiments reported so far have led to different and contradictory assumptions about the electrophoretic migration velocities of ions but none has contributed to the central question of their magnitude.

However, before continuing the discussion of further approaches to determining the ion speeds, initiated by Wilhelm Hittorf and his concept of the transference number, the author will not fail to show that theories long believed to have been overcome still had ardent supporters in the middle of the *Long Nineteenth Century*, albeit rarely. At that time, it came as a surprise that established theories, e.g., those of M. Faraday, were still rejected by staunch proponents of the Phlogiston Doctrine. One of these advocates was William Ford Stevenson (1811 – 1852), after all Fellow of the Royal Society, who in 1846 published a book whose title already fully reveals its content. It reads "*Most important errors in chemistry, electricity, and magnetism, pointed out and refuted: and the phenomena of electricity, and the polarity of the magnetic needle accounted for and explained by a Fellow of the Royal Society*".[23] Moreover, Stevenson was so violently provoked by the award of the Gold Medal of the Royal Society to the noted physico-chemist William Robert Grove[9] in 1847 that he wrote a second conspicuously polemical book in 1849 entitled "*The Composition of Hydrogen and the Non-Decomposition of Water incon-*

[5] The English apothecary, physician and natural philosopher Sir William Watson (London, 1715 – 1787) charged wires with electric machines in London in 1747 and 1748.[12][13] However, differences in the time of appearance of the discharges at the two extreme ties of a wire of 4 miles in length could not be determined visually. Charles Wheatstone attributed this miscarriage to the laggardness of the observing eye. He therefore constructed a device with rapidly rotating mirrors with which time intervals for the occurrence of electrically generated sparks could be measured beyond a millionth of a second. The measured velocity of light was either 576000 or 288000 miles per second, depending on the experimental circumstances assumed.[14] If we take the English mile, standardized in 1592 by the English Parliament as 1609 m, the latter velocity is about 460000 $km.s^{-1}$. Faraday himself attempted to derive an approximate value for the velocity of ions in solution, not of the action of electricity (nrs. 1651. and 1652. in the *Thirteenth Series*). It just ended with a hypothetical comparison of the quantity of electric power equal to the effect appearing instantly at the distance of 576000 miles from its source, on the one hand, with the effect which is obtained by the movement of hydrogen and oxygen after electrolysis of water through a certain distance of one tenth of an inch within an hour and a half, on the other hand.

[6] Our justification for coining the term *electrophoretic current* for the flow of charges carried by ions is given in detail in Part 2.[4] We emphasize that by *electrophoresis* we mean the movement of charged particles under direct current conditions, but not under alternating current conditions. Under both conditions the electrical conductivity nevertheless has the same values.

[7] Claude Servais Mathias Pouillet (1790, Cusance, Doubs – 1868, Paris) was a French physicist, and politician until the February revolution from 1848. His research comprised optics, photometry, thermodynamics and electricity. His main publications were, among others, the book "*Élémens de physique expérimentale et de météorologie*", published in 1827,[17] and "*Mémoire sur la chaleur solaire, sur les pouvoirs rayonnants et absorbants de l'air atmosphérique et sur la température de l'espace*"(*Memoir on the solar heat, on the radiating and absorbing powers of atmospheric air and on the temperature of the space*) in 1838.[18] Claude Pouillet must not be confused with his brother Marcellin Pouillet, who was chemist at *Conservatoire des Arts et Métiers*.

[8] Alfred Smee (1818, Camberwell – 1877, Finsbury Circus) was an English electro-scientist, metallurgist, chemist and surgeon.

[9] Sir William Richard Grove (1811, Swansea, Wales – 1896, London) was a Welsh advocate. He did not practice this job; instead he became interested in electrical phenomena, and published his first paper on this topic in 1838.[24] Grove improved the Voltaic battery and invented a fuel cell battery what he reported in 1839,[25][26] and which is named after him. A basically similar cell[27][28] was invented by the German-Swiss physicist and chemist Christian Friedrich Schönbein (1799, Metzingen – 1868, Baden-Baden).

trovertibly established, in answer to the award of a medal by the Royal Society whereby the Contrary Doctrines are absolutely affirmed, also the Absurdity of the existing Systems of Electricity and Magnetism demonstrated and the True One Given."[29] In this book, which, like his earlier one, was published by the author himself because all journals had refused to accept it, he again vehemently rejected all previous theories about the effect of electricity on liquids because they did not agree with the Phlogiston Doctrine. He was going so far as to accuse the Committee of the Royal Society, which awarded Grove the Golden Medal, of complete incompetence. We briefly quote verbatim one of his numerous attacks on the Committee, for example that on pp. 22-23.

> Every body must know that the appreciation of a paper upon a special subject, such as Chemistry, must *finally* depend upon the opinions and report of those gentlemen who are *presumed* to be specially acquainted with the subject. My observation must therefore be understood to apply exclusively to the COMMITTEE of Chemistry, and to no other portion of the Royal Society. Of the names of the gentlemen composing this committee I am at this moment ignorant, and I have no motive whatever to induce me to make the inquiry.
> I must, however, remark, that a TOTAL ABSENCE OF INFORMATION (a fact which will be elicited from a perusal of these pages) in a conclave of scientific men upon a subject on which they were *voluntarily* about to pronounce, and actually did pronounce, a most important opinion (an erroneous one, as may be supposed) pregnant with vast consequences is, I believe and trust without a parallel in the annals of science.[10]

To remind the readers of the state of science at that time, we mention that already more than four decades ago, *viz*. in 1804, the last prominent supporter of the phlogiston theory died, John Priestley, who defended this doctrine as late as 1803 with the book "*The Doctrine of Phlogiston Established and that of the Composition of Water refuted*".[30] We refer, on the other hand, to some contemporary works, e.g., those of Michael Faraday in 1846,[31] of James Clerk Maxwell´s first paper also in 1846,[32] of Rudolf Clausius and of James Prescott Joule in 1850,[33, 34] to name only the best known, and come to the works of Wilhelm Hittorf, who raised the knowledge of ion migration to a next level.

[10] The capital letters and italics correspond to the original text.

W. HITTORF: RELATIVE MIGRATION VELOCITIES AND TRANSFERENCE NUMBERS OF IONS OF AN ELECTROLYTE

From 1853 to 1859[11] Wilhelm Hittorf developed a promising attempt to measure the migration velocities of ions.[12] He described his basic idea in 1853 in a first paper out of four, entitled "*Ueber die Wanderung der Ionen während der Elektrolyse. Erste Mittheilung.*"[36] (*On the migration of ions during electrolysis. First Notice.*)[13] This concept was still based on the traditional view that a cation, *C,* and an anion, *A,* are tightly bound in solution in the absence of an electric field, forming a single macroscopically uncharged unit or molecule *CA*. Consequently, free ions were believed not to exist. Note that in Hittorf´s conception the distance between the molecules was much larger than the molecules´ size. This differed significantly from the theories of Grotthuß, Davy and Berzelius, in which the molecules in the chains to which they arrange were in direct contact (see Part 2 of our series). This greater distance between the molecules is a precondition for Hittorf´s theory.

Hittorf based his concept on the relationship between the velocity of ions to their respective electrodes and the change in their concentrations there before and after electrolysis. Hittorf believed, in contrast to the established assumption of equal velocities, that ions migrate at different speeds, and postulated that this hypothesis can be confirmed by determining the change of their concentrations in their solutions at the electrodes by chemical analysis. He assumed that more of faster-moving cations must be found at the cathode and fewer of slower-moving anions at the anode, and vice versa.

In Hittorf´s concept the molecules *CA* with cations *C* and anion *A* are initially aligned under the influence of the electric potential, so that the ions are directed towards their respective electrodes, as shown in Fig. 1, row *a* (anions are depicted as black semicircles, cations

[11] On November 24th of the same year 1859 Charles Darwin published his seminal book "*On the Origin of Species by means of Natural Selection, or the Preservation of favoured Races in the Struggle for Life*".[35]

[12] Johann Wilhelm Hittorf (1824, Bonn – 1914) studied mathematics und natural sciences in Bonn. After receiving his doctorate in 1848, he was given the position of a university lecturer in the following year. In 1856 he became professor of physics and chemistry at the University of Münster (Westphalia) and director of laboratories from 1879 to 1889. In the 1850s Hittorf contributed significantly to electrochemistry with the introduction of the transference numbers of ions, and constructed the instrumentations for their determination. Hittorf directed his research further to the passage of the electric current through gases and to the emitted spectra, and discovered the electron rays, later called cathode rays.

[13] The Engl. translation was published in *Harper's Scientific Memoirs* from 1899, ref. [37], pp. 49-80.

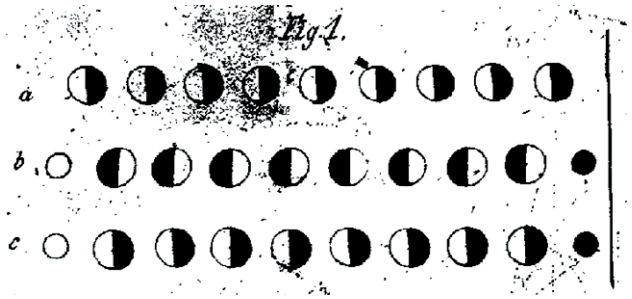

Figure 1. Hittorf´s concept and determination of the relative velocities and the transference numbers for the case that anions and cations have equal migration velocities. Anions: black semicircles; cations: open semicircles. In solution, the ions are present in pairs as macroscopically uncharged molecules, since all ions unite with their oppositely charged ions by electrostatic attraction. There are no free ions present. Row *a*: When an electric potential is applied, the molecules are aligned in the direction of their respective electrodes. Row *b*: The molecules are split into their ions, which migrate towards their corresponding electrodes, but reassemble with the oppositely charged ions in their immediate vicinity to form new molecules. Row *c*: The molecules rotate 180° to take the proper position in the field. Details can be found in the text. The figure is reproduced from Hittorf´s publication from 1853 (ref. [36], Plate II, after p. 352).

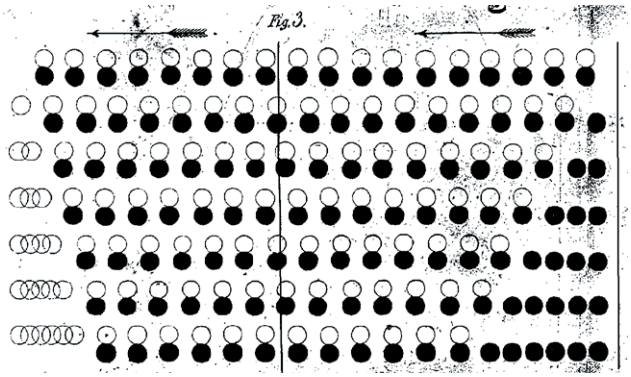

Figure 2. Hittorf´s concept and determination of the relative velocities and transference numbers for the case that anions and cations have different migration velocities. Anions: black full circles; cations open circles. Cations migrate in direction of the arrows to the left, anions to the right. Details are described in the text. (From ref. [36]).

as open semicircles; single-charged ions are considered). Next, the assembled ions of molecule *CA* separate from each other and move to the nearest oppositely charged ion,[14] with which they combine to form a new molecules *AC* (row *b*). Note that the ion travels as a free ion during the movement over this distance. Prior to the next step, this newly formed molecules *AC* had to rotate by 180° from *AC* to *CA* in order to get their proper position, that is to say, to be aligned with the respective electrodes (row *c*). Then, this process of decomposition and recombination continues as long as the electric potential is applied and the ions are finally decomposed electrolytically. The number of cations and anions decomposed by electrolysis is the same, independent of their velocities and in accordance with Faraday´s law of definite electrochemical action (see Chapter 3.1.4. in Part 2).

In order to prove the validity of his concept experimentally, a vessel of a measuring device was first filled with a solution with a given initial concentration of the electrolyte. In contrast to Daniell´s instrument, the vessel was not separated by a membrane, a diaphragm or another porous boundary. In Figure 1 the ions of the molecules are depicted as full and empty semicircles. If we suppose that the velocities of both ion species are equal, the ions reassemble into new molecules in the middle of their distance, and both travel along the same distance in a given time. Therefore, the change of their concentrations in their respective electrode compartments would be the same.

In the general case, when the two types of ions have different velocities, they travel different distances within a certain time before reuniting to form the next molecule. Then, eventually, more ions of the faster species reach the solution near the respective electrode, and fewer ions of the slower species reach the oppositely charged electrode. Thus, the number of ions of the two species in their electrode compartments is unequal. We emphasize again that this argument is valid even if, according to Faraday's above-mentioned law, the same number of both ions is electrolytically decomposed in their compartments.

The question arises as to how Hittorf solved the problem of separating the vessel into an anode and cathode compartment without inserting a porous boundary. Hittorf's solution, which circumvented this problem is shown schematically in Figure 2.[15] There, the electrolyte solution is separated not by a real boundary but by an imaginary one, indicated by the vertical line, which can be placed anywhere. The cathode is on the left side,

[14] We called it counterion in previous Part 2, and will use this term in our publications. It is customary in modern phraseology.

[15] In this figure, the representation of the molecules in the vertical direction and in direct contact to each other is as in Berzelius' paper, ref. [38], p. 278, is in discrepancy with Hittorf´s own assumption on p. 180, ref. [36] "*Der Zeichnung liegt die Annahme zu Grunde, daß die Entfernung zwischen den benachbarten Atomen des Elektrolyten größer als diejenige ist, in welcher die chemisch verbundenen Ionen jedes Atoms von einander abstehen*" (*The drawing is based on the assumption that the distance between the neighboring atoms of the electrolyte is greater than the distance between the chemically bonded ions of each atom.*) This requirement is satisfied in Figure 1. However, the deviation in Figure 2 has no consequence for Hittorf´s conclusions. [The author].

the anode on the right side. The cations are depicted as open circles, the anions as black circles. The transfer mechanism involving the 180° rotation is the same as described above. In the present example, the initial number of each ion type or equivalent is 18 (top row), The fictitious cathode compartment (left) contains 8 molecules, the anode compartment 10.

After completing the circuit, the ions migrate with velocities, *v*. In the case depicted in Figure 2 the cations cross the fictitious boundary faster than the anions. We assume, for example, that the anions travers ⅓, the cations ⅔ of their distance after their dispartment and the following recombination. But how can these different distances be determined? Hittorf turned this problem from the molecular to the molar scale, and developed the following method, for which we select at random the conditions in the anode compartment. He considered the change of the numbers (or equivalents) of anions and cations there. He recognized that from the begin of the electrolysis, shown in the top row, to its end (in the bottom row) the number of cations is reduced from 10 to 6, that is, 4 cations traversed the boundary and migrated towards the cathode. The number of anions in the anode compartment changed from 10 to 12, that is, it increased by 2. In the solution, 6 from the 12 anions remained and 6 were electrolytically decomposed; this decomposition applies to the cations as well. Thus, 2 anions crossed the boundary and moved into the anode compartment. To conclude, in the anode compartment a reduction of cations from 10 to 6 happened, equals 4, and the change of the number of anions is from 10 to 12, equals two. It follows that the change in equivalents in the anion compartment for the anions and for cations is ⅓ and ⅔. This result which would be obtained by experiment is identical to our input assumption.

Generally speaking, if the one ion traverses 1/*n* of the distance, the other (*n*-1)/*n*, after electrolysis the compartment of the former ion contains 1/*n* equivalent more of the one, and (*n*-1)/*n* equivalents less of the other ion. Hittorf termed these numbers "Überführungszahlen" (transference or transport numbers) which we symbolize by τ. Their sum ($\tau_+ + \tau_-$) equals one. As mentioned above, they can be derived by quantitative chemical analysis of their concentrations.

The transference numbers express the migration velocity of the one ion relative to the counterion in a given electrolyte. The smaller the transference number of the ion, the slower is its migration velocity relative to its counterion. In the above example τ_- was ⅓, τ_+ was ⅔, which is consistent with our initial assumptions about the different distance the two ions travel before forming a new molecule, or that the cation migrates at twice the speed of the anion. In general, the ratio of the transference numbers is equal to the inverse ratio of the velocities by $\tau_+/\tau_- = v_-/v_+$. It should be noted that one does not obtain the absolute migration velocity of an ion, but only that relative to the counterion of the same electrolyte.

It is also mentioned that the transference number expresses the fraction of current, *i*, transported by one type of ion in relation to the total current, i.e., the sum of the currents of both types of ions. It reads, for example, for the cation $\tau_+ = i_+/(i_+ + i_i)$. To get the currents Hittorf used a Poggendorff silver electrometer to simultaneously measure the total amount of current flowing during electrolysis and obtained ($i_+ + i_i$). Then, he recalculated i_+ from the number of equivalents of the cation which he determined by chemical analysis in the anode compartment, and received the fraction of the current which was transported by the cation, and which is equal to its transference number τ_+. Since ($\tau_+ + \tau_-$)=1, the transference number, τ_-, of the anion can be calculated without measuring it. It is therefore sufficient to measure the concentration, and thus the value of τ, of only one ion in one compartment, and neither both in the same compartment, nor all four in the two compartments.

For the experimental determination of the concentrations of the ions after electrolysis, Hittorf modified the cell of Daniell and Miller by omitting the membranes. Readers interested in details are referred to the comprehensive description in Hittorf´s *First Notice* of 1853[36] which after all covers the three pages 187-189. The schematic drawing of the device is given in Fig. 4 in Plate II, after p. 352.

In this *First Notice* Hittorf examined different copper and silver salts. He found that their transference numbers were independent of the (low) current. Their changes, within the investigated temperature range between 4°C and 21°C proved to be unreliable.[16] Relevant for future research, especially for that of Friedrich Kohlrausch, was his observation that the transference numbers can depend significantly on the concentration of the electrolyte.

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[16] Note that we are discussing transference numbers, not conductivities. The German physicist Gustav Heinrich Wiedemann (1826, Berlin – 1899) observed that the electric conductance (or resistance) of electrolyte solutions depends on their viscosity. He varied the viscosity of the solution by varying the temperature and/or the salt concentration, and supposed a relation between the electric behavior and the mechanistic properties of the solutions. However, Wiedemann found that these conclusions were based on imperfect and preliminary experiments (see ref. [39], pp. 169, 170). Wiedemann´s conclusions nonetheless preceded Walden´s rule or Walden´s product (named after Paul Walden, a Russian-Latvian-German chemist), which he formulated in 1906,[40] sixty years after Wiedemann´s assumptions. Walden´s rule states that the product of the conductivity of an electrolyte solution at limiting conditions and the viscosity of the solvent is constant and independent of the solvent.

Hittorf also speculated on possible sources of error, primarily with respect to the role of water, since he assumed, contrary to popular belief, that instead of the cation of the salt, H^+ from the water could be reduced directly. However, he found that this primary, direct reduction of H^+ to gaseous hydrogen could be neglected. To support his opinion, he used solvents that were not decomposed electrolytically and measured the transference numbers in absolute ethanol and, in later Notes, in amyl alcohol. His results made it clear that water did not contribute to the current; it acted only as an electrochemically inactive solvent of the salts.

For the *Second Notice* from 1856 Hittorf constructed an improved device, again without membrane, and investigated whether or not electroosmotic flow affected the transference numbers.[41] He compared the results measured in devices with and without a membrane, and obtained the same transference numbers.

History showed that it is not uncommon for a front of critics and opponents to form when a new theory or method is introduced.[17] This also happened with Hittorf, and so he began his *Third Notice* in 1859[42] with a reply to Wiedemann´s critique.[39] Then a comprehensive reply to the derogatory reproaches [43, 44] of Gustav Magnus[18] followed, whom he called "Hauptgegner meiner Arbeiten" (main opponent of my works). In ref. [42], p.342, Hittorf wrote

> Magnus hatte in seiner ersten Abhandlung, §9, meiner elektrischen Arbeiten erwähnt und angegeben, daß dieselben mit der Zersetzung, welche die Elektrolyte durch den Strom erfahren, nichts zu thun hätten und über die Daniell´sche Theorie … nichts lehren. (Magnus had mentioned my electrical work in his first paper, §9, noting that it had nothing to do with the decomposition of electrolytes by the current and that it taught nothing about Daniell's theory.)

The rigor with which Hittorf rejected this criticism is evident in the fact that it extended over twenty printed pages. The remaining part of this *Third Notice* dealt with the transference of compounds that Hittorf had not examined in the *Second Notice*.

A paragraph in the *Fourth* and last *Notice*[45] worth noting comprised, beyond the determination of transference numbers, Hittorf´s critique of Clausius´ theory.[46] We will return to this theory below. Contrary to established opinion, Clausius postulated that free ions do exist in solution even in the absence of an electric potential due to their motion caused by their thermal energy. Hittorf strictly rejected this theory and commented on p. 584

> Der Schluß, zu den Hr. Clausius aus seinen Prämissen gelangt, steht meinen Erfahrungen nach mit der Wirklichkeit im Widerspruch, … Es sind bloß die letztgenannten Zersetzungen, welche die Theorie der Elektrolyse von Hrn. Clausius brauchen kann. Bis jetzt ist kein Chemiker so kühn gewesen, sie anzunehmen, … (The conclusion Mr. Clausius draws from his premises is, in my experience, at odds with reality, … Only the latter decompositions can use Mr. Clausius' theory of electrolysis. Until now, no chemist has been so bold as to accept it.)

We see that Hittorf was not only the target of criticism, but was himself a critic. The last part of this paper dealt with the determination and discussion of the transference of complex ions, and what he called "double salts".[19] Salts of cadmium and iodine, for example, which contain complex anions will play an important role in Arrhenius´ dissociation theory.

At the time of Hittorf publications transference numbers by his method were also determined by G. Wiedemann[47] and by A. Weiske.[48] These and Hittorf´s data were later used by Friedrich Kohlrausch in his studies of conductivities.

We already pointed out that a given ion has different transference numbers in electrolytes of different composition, depending on the counterion. For example, Hittorf determined τ_+ of 0.63 for Ag^+ as acetate and of 0.47 as nitrate (ref. [36], p. 206).[20] From these data it follows that the silver ion velocity is 1.7 times higher than that of acetate, and about 10% lower than the nitrate velocity. Regrettably, the method provided only the relative ion velocities, but not the absolute values of the individual ion species. The decisive advance towards the solution of this problem was initiated by Kohlrausch's work on the conductivities of electrolyte solutions, in particular by his law of independent ion migration. We will discuss it below.

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[17] Svante Arrhenius, for example, was even lampooned when he presented his dissociation theory. Ironically, 25 years later he was awarded the Nobel Prize. We will tell this episode in the next Part 4 of our historical review series.

[18] Heinrich Gustav Magnus (1802, Berlin – 1870) was an influencing German chemist and physicist. In 1845 he became professor of physics, chemistry and technology at the University in Berlin. His students or coworkers were, among others, A. von Baeyer, E. Du Bois-Reymond, R. Clausius, P. von Groth, H. von Helmholtz, G. Kirchhoff, A. Kundt, E. Sarasin, J. Tyndall and E. Warburg.

[19] It can be assumed that the majority of readers have little interest in details. We have therefore included them in this footnote. These double salts consisted, for example, of *K*, *Ag*, and *CN*, or *Na*, *Pt*, and *Cl*. Their number was rather limited, since most were not stable when dissolved in water. Hittorf measured and discussed the transfer, for example, of the complex salts consisting of cadmium and iodine, which he was able to dissolve undecomposed in absolute ethanol and in amyl alcohol.

[20] These values are in good agreement with current transference numbers, at 25°C, e.g., of 0.624 for Ag^+ (0.01 mol.L^{-1}) as acetate and of 0.4648 as nitrate.[49]

The reader may wonder that this historical review does not even mention a certain empirical law which – as generally assumed – Friedrich Kohlrausch had confirmed by careful measurements in 1869.[50] It had, however, already been applied earlier, namely in the 1830s by Michael Faraday and in the 1850s by Wilhelm Hittorf in their investigations of the electrophoretic current in solutions of ions. It was also an indispensable law in further research on this subject and is still daily practice. What is meant is the law about the connection between electrical potential difference, resistance and current strength, which Georg Simon Ohm had found empirically in the middle of the 1820s, albeit for metallic wires, i.e., for 1st class conductors.[51-56] That it also applied to 2nd class conductors, that is to say, to solutions of ions, was by no means a foregone conclusion because of the fundamental difference in the nature of conduction. So how did it come about that Ohm's law proved to be valid also for solutions of ions?

A LEAP IN TIME, BACK TO 1831: G. TH. FECHNER´S PROOF THAT OHM´S LAW APPLIES TO SOLUTIONS OF ELECTROLYTES

Before we get to Kohlrausch´s above-mentioned conductivity measurements, we have to realize that he did, in principle, measure the resistance or the specific resistance of the electrolyte solution, a property that we have yet not even addressed in this historical retrospect. To do this, we have to go back to the 1820s, to the time when Faraday made experiments in organic chemistry in Davy´s laboratory. At that time Georg Simon Ohm pioneered the research in electric resistance of 1st class conductors.

Ohm was not affiliated with the scientific community at the time of his research. He was a school teacher at the Jesuit Gymnasium in Cologne when he derived his law in mid-1820s. He performed his experiments in the laboratory of this college with wires made of different metals and various lengths and diameters. He published his preliminary results initially in two treatises in 1825.[51, 52] As he was not satisfied with his work,[21] he developed a new theoretical approach, which led to the law named after him. This well-known law says that the current strength is directly proportional to the electric potential difference and indirectly proportional to the resistance of the conductor. Ohm published his new theory in 1826.[53-55] In his *opus magnum* "*Die galvanische Kette, mathematisch bearbeitet*" (*The galvanic chain, mathematically processed*) [56] he summarized in 1827 the results together with a comprehensive mathematical treatment.

Ohm´s work did not attract much attention, but the interest changed when French physicists claimed priority of the law for Claude Servais Mathias Pouillet,[22] who rediscovered Ohm´s law years later and published his results in 1837[57] (German version in ref. [58]; the editor, Johann Christian Poggendorff, added a critical comment to this version, cited in footnote [23]). Pouillet derived the according relations rather from experimental results which was considered as being superior to Ohm´s merely theoretical approach.

His assertion of priority provoked a heated debate, led primarily by Jean Claude Eugène Péclet, who published a harsh rebuff of Pouillet's claim in an essay entitled "*Lettre touchant un passage de la dernière édition du Traité de Physique de M. Pouille*t" (*Letter concerning a passage in the last edition of the Traité de Physique by M. Pouillet*)[61] stating

> C'est seulement en 1837 que M. Pouillet a publié son Mémoire dont toutes les formules se déduisent de celles de M. Ohm par de simples transformations, comme M. Henrici l'a démontré (Annales de Poggendorff tomes LIII[62] et LIV[63]), … (It was not until 1837 that M. Pouillet published his Memoir, all the formulas of which can be deduced from those of M. Ohm by simple transformations, as M. Henrici has shown (Pogg. Annal. LIII and LIV),…)[24]

Finally and at last Ohm's priority was recognized internationally, and in 1841 he received the prestigious Copley Medal from the Royal Society in London. Nevertheless, in Germany it lasted until 1852 that he became professor for experimental physics at the University in Munich, at the age of 63, two years before his death.

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[21] In the last two sentences at the end of his second paper from 1925,[51] Ohm complained about the lack of support for his research: "*Meine Arbeit fängt allmählig an, sich zu einem Ganzen zu runden. Nur bedaure ich, daß ich häufig aus Mangel an Mitteln, Untersuchungen abbrechen muß, die ich so gern weiter verfolgt hätte*" (*My work gradually begins to round off into a whole. My only regret is that I often have to abandon investigations that I would have liked to pursue further, due to lack of funds.*)

[22] We quoted Pouillet already in the Introduction of this review.

[23] Poggendorf commented: „*Wiewohl die Resultate dieser Abhandlung zum Theil nur Bestätigungen der von Ohm („Die GaIvanisehe Kette" und Schweigg. Journ. Bd. XXXXVI S. 137),*[59] *entdeckten, auch von Fechner (Maaßbestimmung über die galvanische Kette) und früher von Pouillet selbst (Annal. Bd. XV S. 91)*[60] *beobachteten Gesetze sind, so schien doch die unverkürzte Mittheilung derselben,schon weil sie so wenig berücksichtigt wurde sind, nicht überflüssig. P.*" (*Although the results of this treatise are partly only confirmations of the laws discovered by Ohm ("Die GaIvanisehe Kette" and Schweigg. Journ. Bd. XXXXVI p. 137),*[59] *also by Fechner (Maaßbestimmung über die galvanische Kette) and earlier by Pouillet himself (Annal. Bd. XV p. 91)*[60] *so the unabridged communication of them seemed not superfluous, already because they were so little considered. P.*) [citations added by the author].

[24] Complete literature sources added by the author.

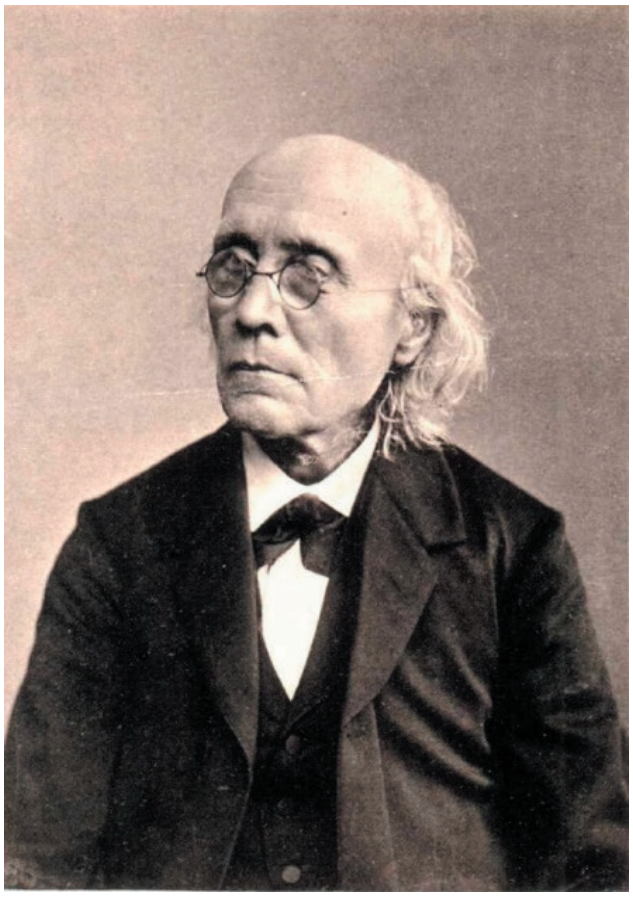

Figure 3. Portrait of Gustav Theodor Fechner (1801 – 1887). Date and author unknown. Source, ref. [64], public domain.

Initially regarded of minor importance, his law was later recognized as highly significant for the technology of ever-widening electrical telegraphy, especially after the mid-1850s with the introduction of the single-wire system by Samuel Morse.

We have mentioned above that after his publication in 1827 Ohm´s work was widely ignored, and only few scientists recognized its importance, with Gustav Theodor Fechner[25] as Ohm´s main supporter (his portrait is shown in Figure 3).

Fechner's scientific merits should not be underestimated, although they were, because he extended Ohm's theory of the 1st to 2nd class conductors. His work is therefore indispensable for electrophoresis. He is also worth noting because, in addition to his scientific interests, he had a penchant for writing satirical pseudo-scientific works. In 1821 he published his first paper ever; it was entitled "*Beweis, daß der Mond aus Jodine bestehe*" (*Proof that the moon consists of iodine*),[65] published under his pseudonym Dr. Mises. It is a humorous pamphlet against materialistic medicine. Of similar kind was his scientific satire from 1825 "*Vergleichende Anatomie der Engel*" (*Comparative anatomy of angels*).[66]

In these early 1820s, Fechner prepared the translation of Jean-Baptiste Biot´s textbook "*Précis élémentaire de physique expérimentale*"[67, 68] into German, which appeared in 1824 and 1825, but he found the description of electricity and of the theory of galvanism in this book highly out of date. In order to raise these themes to the contemporary level, he decided to upgrade it by Ohm´s theory. At this occasion, he found keen interest in this topic, and expanded his research from the hitherto investigated metallic conductors to liquids, pioneering in this way the quantitative context of electric potential difference, current and resistance in ion solutions.[26] After two years of extensive experimental work, Fechner presented his results and conclusions in the book "*Massbestimmungen über die galvanische Kette*" published in 1831.[69]

Fechner demonstrated that Ohm´s law not only applies to metallic conductors, but also to electrolyte solutions. He gave a résumé of these findings (amongst others) by stating on pp. 235-236 of his book

> … (i) Der Leitungswiderstand der Flüssigkeiten ist dem Abstande der Erregerplatten darin direct proportional… (ii) Der Widerstand der Flüssigkeit steht im umgekehrten Verhältnisse des Querschnittes der Flüssigkeit… .(iii) Der Widerstand der Flüssigkeit ist unabhängig von Beschaffenheit der Plattenpaare… (iv) Der Widerstand der Flüssigkeit nimmt ab mit der Quantität saurer oder salziger Bestandtheile, die man in dieselbe hinzufügt. (v) Durch fortgehends gleiche Zumischungen gleicher Antheile Säure wird der Widerstand des Brunnenwassers auch fortgehend um gleiche Quantitäten verringert…" ((i) The electric resistance of the liquids is directly proportional to the distance of the excitation plates therein. (ii) The resistance of the liquid is in inverse proportion to the cross section of the liquid … (iii) The resistance of the liquid is independent of the nature of the plate pairs … (iv) The resist-

[25] Gustav Theodor Fechner (1801, Groß-Särchen, now Żarki Wielkie, near Muskau in Lower Lusitia – 1887, Leipzig) attended the Academy of Arts in Dresden from 1814, and studied medicine there from 1817 and in Leipzig from 1818, where he graduated in 1823. However, he left the field of medicine and completed his habilitation in 1823 at the Philosophical Faculty. He was appointed professor of physics in 1834; his research focused on galvanism and optics. Due to a severe eye complaint from 1840 to 1843, he retired. In 1860 he expanded Weber´s law to Weber-Fechner law. This law expresses the non-linear relationship between psychological sensation, S, and the physical intensity, I, as $S = K \ln I$.

[26] The water Fechner used in his experiments can be considered rather as a dilute electrolyte solution. Water with the highest purity which was available for him was well water, which certainly contained a relatively high amount of ions. All the more his results are to be appreciated.

ance of the liquid decreases with the quantity of acidic or salty components are added to it. (v) By continuously adding equal amounts of acid, the resistance of the well water is also continuously reduced by equal quantities…)

If we convert these formulations into a modern notation, we get for a given solution that (i) $R=prop\ l$; (ii) $R=prop(1\text{-}A)$; (iii) $R\neq f(U)$; (iv) and (v) $R=prop(\text{-}S.c)$. Symbol R stands for the electrical resistance of the liquid, l is the distance between the electrodes, A is the cross sectional area of the electrodes, U is the electrical potential difference, and c is the concentration of acidic or salty additives to water; S is a factor of proportionality. It is seen that these findings actually represent the first evidence that Ohm´s law also applies to liquid conductors.[27] It is worth noting that the combination of (i) and (ii) leads to equation $R=prop(l/A)$, in accordance with modern notation as $R=\rho l/A$, in which ρ is termed electrical resistivity or specific electrical resistance.

After his retirement, Fechner focused mainly on philosophy and topics of psychophysics and psychological aesthetics. During this time he published works mainly based on *Natur Philosophie* and religious-mystical ideas.[28]

R. CLAUSIUS' THEORY: FREE IONS ARE PRESENT IN ELECTROLYTE SOLUTIONS EVEN WITHOUT AN APPLIED ELECTRIC FIELD

Coming back now from Fechner´s time to 1852, when Rudolf Clausius[29] turned his interest on 2nd class conductors after publishing his paper about the work done on 1st class conductors and the heat generated thereby[30,[84-86]] In "*Ueber die Elektricitätsleitung in Elektrolyten*" from 1857[46] (Engl. vers., *On the Work performed and the Heat generated in a Conductor by a Stationary Electric Current*,[87] French vers., *Mémoire sur la Chaleur dégagée par les courants électriques*.[88]), Clausius expressed his rejection of the established view of the transport of electric charges through electrolyte solutions, a theory that was going back to Theodor von Grotthuß. Even in Clausius´ time (and later), it was generally assumed that the electrolyte molecules, called the *complete molecules* by him, were composed of atoms or groups of several atoms, which he called *partial molecules*.[31] Each molecule consists, as was believed, of two tightly bound partial molecules of opposite charge (i.e., ion and counterion). We recall that in the theories of Grotthuß and Davy the molecules form a continuous chain that extends without gaps from one electrode to the other. Only the terminal molecules of this chain are in direct contact with the electrodes and are the first to be decomposed during electrolysis. Then, an instantaneous recombination of the ions with the counterions of the neighboring molecules, and the decomposition of the newly formed molecules happens along the entire chain. We will not repeat this mechanism of the flow of electricity as we discussed it in detail in the previous Part 2 of our series.[4] Important to emphasize is that the established theories of Grotthuß and Davy exclude the presence of free ions in solution in the absence of an electric potential, because an electric force would be mandatory to separate the tightly bound ions of a molecule.

In his said paper from 1857 Clausius radically broke with this conventional view[46, 87, 88] He first considered a liquid that contains "electrolytic molecules", i.e., electrolytes. Clausius' basic condition for all further considerations was that electric neutrality had to be in every volume element of the liquid. If there was no external electric force, the molecules were dispersed in an arbitrary order and possibly vibrated around a certain equilibrium position. He further assumed that in the same molecule the attraction between ion and counterion, which are close to each other, is greater than the attraction of one ion to the counterion of another molecule.

Clausius assumed, in analogy to his paper "*Ueber die Art der Bewegung, welche wir Wärme nennen*"[90] (*The nature of the motion which we call heat*)[91] from

[27] It is clear that points (iv) and (v) do not have counterparts in pure metals, but the observations were nevertheless correct and preceded at least within a small concentration range the measurements of the conductivities by F. Kohlrausch.

[28] Fechner published, for example, poems,[70] a book about *the soul life of plants*,[71] 3 vol. about *things of heaven and the hereafter*,[72][73][74] about psychophysics,[75][76][77][78] a book with the mysterious title *"the day view versus the night view"*,[79] but also a paper in Pogg. Ann. Phys. Chem. "*Ueber die Bestimmung des wahrscheinlichen Fehlers eines Beobachtungsmittels durch die Summe der einfachen Abweichungen*" (*On the determination of the probable error of an observation mean by the sum of the simple deviations.*)[80]

[29] Rudolf (Julius Emanuel) Clausius (1822, Köslin, province of Pomerania in Prussia, now Koszalin, Poland) -1888, Bonn] is one of the first theoretical physicist of the Nineteenth century and a central founder of thermodynamics (2nd law of thermodynamics), and the kinetic gas theory. He created the concept of entropy and contributed significantly to fundamental concepts of statistical mechanics. Less known is his work on free ions in solutions, which he carried out mainly during his research activities as professor at the ETH in Zürich in the 1850s.

[30] In this paper Clausius derived the theoretical basis of the empirical results of J. P. Joule,[81] M. E. Lenz, the father of Robert Lenz[82] and Edmond Becquerel.[83]

[31] Clausius did not use the terms ion, anion and cation in this paper, although they have been known since 1834, when Michael Faraday introduced them.[89] Clausius differentiated the neutral "*Gesamtmolecüle*" (*complete molecules*), which were combinations of the oppositely charged ions, which he called "*Theilmolecüle*" (*partial molecules*). For the sake of simplicity, we name Clausius´ "*complete molecule*" as molecule, and, "*partial molecules*" as ions, and cations and anions if appropriate.

the same year that – in absence of an electric field – the electrolyte molecules do not remain permanently in the liquid in an equilibrium position around which they oscillate. On the contrary, they are moving and their motion is irregular. Moreover, an ion can be disconnected from its molecule due to its thermal energy and then moves randomly between the other molecules in the solution. It can happen that this ion attracts the counterion of another molecule more strongly than the ion and counterion of this molecule attract themselves. In this case, this ion and the counterion of the molecule form a new molecule and the parent ion is set free. It then moves in the solution between the other molecules and reacts with one of them in the manner just described; then the whole process continues.

A second possibility for the formation of free ions assumed by Clausius was that two different molecules interact with each other. In this case, it can happen that the ion of one molecule takes a preferred position over the counterion of the other molecule and these two ions combine to form a new molecule. This process releases two ions, which either combine to form a new molecule or disperse between the other molecules in the solution and act there as described above. We emphasize again that we have described the situation in the absence of an external electric force. It was considered by Clausius as the natural state of electrolytes in solution.

When an external electrical force acts, the first reaction of the molecules would be to rotate into the proper position towards their respective electrodes (what Davy and Berzelius did not take into account, but Hittorf did). Then the force would separate ion and counterion of a molecule and drive them in opposite directions. In this case the ion of one molecule may combine with the counterion of the other. However, this can only happen if the attractive force between the ion and the counterion of the one molecule is overcome. Clausius argued that the influence of the external electric force not only starts from a certain strength, but that even the slightest force acts in the manner described above. The magnitude of the effect increases with the strength of the force. That is, the effect fully complies with Ohm's law. He wrote (we reproduce verbatim the Engl. version, ref. [87], p. 99-100)

> But in order to separate the already combined partial molecules, the attraction which they exert upon each other must be overcome, for which a force of a certain intensity is necessary; hence we are led to the conclusion, that so long as the force acting within the conductor does not possess this requisite intensity, no decomposition whatever can take place; but that, on the other hand, as soon as the force has attained this intensity, a great many molecules must be simultaneously decomposed, inasmuch as all are exposed to the influence of the same force, and have almost the same relative positions to each other.

and continued

> So long as the moving force acting within the conductor is below a certain limit, it causes no current whatever; so soon as it attains this limit, however, a very strong current is suddenly produced. *This conclusion, however, is in direct contradiction to experiment*. The smallest possible force gives rise to a current accompanied by alternate decompositions and recombinations, and the intensity of this current increases in proportion to the force, according to Ohm's law.

However, in the original paper written in German, this passage has another meaning; it reads (ref. [46], p. 346-347)

> So lange die im Leiter wirksame treibende Kraft unter einer gewissen Gränze ist, bewirkt sie gar keinen Srom, wenn sie aber diese Gränze erreicht hat, so entsteht plötzlich ein sehr starker Strom. *Dieser Schluß widerspricht aber der Erfahrung vollkommen*. Schon die geringste Kraft bewirkt einen durch abwechselnde Zersetzungen und Wiederverbindungen geleiteten Strom, und die Intensität dieses Stromes wächst nach dem Ohm'schen Gesetze der Kraft proportional. [The reader's attention is drawn to footnote [32]].

If an external electric force acts, the cations and the anions in the solution do not move randomly, but are driven in opposite directions. In addition, the force facilitates the decomposition of the molecules, and the detachment of an ion from its molecule occurs more frequently than without such electric force. Clausius assumed that the number of positive and negative ions which move in opposite direction is not necessarily equal, it depends on their activity of molecular motion, which can be different for different ions. The counter-directed movement of both types of ions represents the galvanic current[33] in the liquid. Its strength is given by the sum of the flows of both ions.

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[32] The author would like to add a note here. He refers to the misleading or even wrong translation of Clausius' original work in the English version, which he has marked in italics in the main text. Clausius wrote verbatim: "*Dieser Schluß widerspricht aber der Erfahrung vollkommen*." The English version reads: "*This conclusion, however, is in direct contradiction to experiment*." The term *Erfahrung* is translated here by *experiment*, but the correct translation is *experience* – in fact a crucial difference. In the French version the sentence is correctly translated as "*… En d´autres termes, aussi longtemps que la force est au-dessous d´une certaine limite, il n´y a pas de courant, et dès que cette limite est dépassée, il nait subitement un courant intense. Cette conclusion est évidement contraire à l´expèrience.*", ref. [88], p. 254.)

[33] We call it, as defined in the previous parts of our review, the electrophoretic current. It is not the flow of charges carried by electrons. The

This theory also offers the explanation why the conductivity of a 2nd class conductor increases with increasing temperature: it is because the greater the activity of motion of the particles due to their thermal energy in the solution, the more the decomposition of the molecules is facilitated.

Although Clausius' innovative theory of the permanent presence of free ions offered new explanations for the properties of electrolyte solutions and became widely known, it is noteworthy that the majority of the scientific community (but not its elite) did not replace the theory published by Grotthuß half a century ago in 1805. This lack of scientific acceptance was criticized, to quote an arbitrarily chosen example, in an 1879 publication, another two decades after Clausius' work. Franz Exner,[34] professor of physics at the University of Vienna and teacher of two Nobel Laureates, pointed out in his publication "*Ueber die Electrolyse des Wassers*" (*On the electrolysis of water*), ref. [92], p. 350)

> … dass die Clausius'sche Hypothese über die Constitution der Flüssigkeiten der Theorie der Electrolyse… mit den bekannten Thatsachen nicht nur nicht im Widerspruche steht, sondern durch dieselben auf das Entschiedenste bestätigt wird. Unter allen Umständen aber ist es an der Zeit, die jetzt noch fast ausschliesslich citirte Grotthuss'sche Theorie der Electrolyse endgültig fallen zu lassen; denn seit wir … einen klarern Einblick in das Wesen dieser Vorgänge erlangt haben, erscheinen die von der Grotthuss'schen Theorie geforderten, von Molecül zu Molecül fortschreitenden Zersetzungen und Wiedervereinigungen als eine absurde Vorstellung. (… that Clausius' hypothesis of the constitution of liquids does not contradict the theory of electrolysis … not only with the known facts, but is most decidedly confirmed by them. Under all circumstances, however, it is high time to finally abandon Grotthuß´ theory of electrolysis, which is almost exclusively cited; for since we … have gained a clearer insight into the nature of these processes, the decompositions and reunifications which progresses from molecule to molecule, as required by Grotthuß´ theory, appear to be an absurd notion.)

It took nevertheless another decade before the existence of free, non-associated ions, as formulated in Svante Arrhenius´ seminal theory, was accepted, albeit reluctantly and against fierce opposition.

charges are carried – notabene – by ions, which is the unique feature of electrophoresis.

[34] Franz Serafin Exner (1849, Vienna – 1926) was an Austrian physicist. In 1891 he became professor of physics at the University of Vienna and succeeded J. J. Loschmidt. His best-known students were the Polish physicist Marian Smoluchowski, and the Austrian physicists Erwin Schrödinger (Nobel Laureate for physics 1933), and Victor Franz Hess (Nobel Laureate for physics 1936).

F. KOHLRAUSCH'S FUNDAMENTAL CONTRIBUTIONS TO ION CONDUCTIVITY, VELOCITY AND MOBILITY

Friedrich Kohlrausch,[35] who coined the subject of ion conductivity and ion migration (of strong elecrolytes) in the next decades, concluded his habilitation in 1863, not in electrochemistry, but "*Ueber die elastische Nachwirkung bei der Torsion*"[93, 94] (*Elastic aftereffects during torsion*) at the University of Göttingen, where he became private docent in 1866 and in 1867 associate professor. During the four years in Göttingen from 1866 to 1870 he was mainly, though not exclusively, engaged in research on geomagnetic and electrical measurements, and in investigations of the resistance and conductivity of electrolyte solutions.[36]

Kohlrausch's Entry into Electrochemical Research in 1868

Together with his coworker and former doctoral student Wilhelm August Nippoldt[37] Kohlrausch published his first contributions to electrochemistry in 1868 and 1869 in various versions.[50, 105-107] The overarching subject of these papers was "*Ueber die Gültigkeit der Ohmschen Gesetze für Elektrolyte und eine numerische Bestimmung des Leitungswiderstandes der verdünnten Schwefelsäure durch alternierende Ströme*" (*On the validity of Ohm's laws for electrolytes and a numerical determination of the electric resistance of dilute sulfuric acid by alternating* currents).[106, 107] In this paper, Kohlrausch and Nippoldt posed three questions: (1) How can the influence of the polarization of electrodes be exclud-

[35] Friedrich Kohlrausch (1840, Rinteln-on-Weser – 1910) was a German physisist. He studied physics in Erlangen and Göttingen, where he received his doctoral degree in 1863. In 1867 he was appointed to a professorship in Göttingen. Famous for his exact experimental work, his accurate measurements and his outstanding level in research, Kohlrausch became professor at ETH Zurich in 1870, at Technical University Darmstadt in 1871, at the University of Würzburg 1875, and at the University of Strasbourg as from 1888. He was successor of H. von Helmholtz at the Physikalisch-Technischen Reichsanstalt in Berlin, where he was director between 1895 and 1905. The Reichsanstalt was founded in 1887 and was the first government-financed research institution worldwide, where their staff members – in contrast to the Universities – were not committed to teach. Prominent members were Walther (Hermann) Nernst (1864 – 1941), Emil (Gabriel) Warburg (1846 – 1931), Walther (Wilhelm Georg) Bothe (1891 – 1957), Albert Einstein (1879 – 1955) and Max (Karl Ernst Ludwig) Planck (1858 – 1947).

[36] Beside these main topics he also dealt, e.g., with the construction of an apparatus which serves for room cooling,[95] the speed of propagation of the stimulus in the human nerves,[96] elastic effects after torsion,[97][98] geomagnetic observations in Göttingen,[99][100][101][102] the amount of electricity generated by an influence machine,[103] and others.

[37] The title of Nippoldt´s dissertation was "*Untersuchungen über den galvanischen Widerstand der Schwefelsäure bei verschiedenen Konzentrationsgraden*" (*Studies on the galvanic resistance of sulfuric acid at various degrees of concentration.*)[104]

ed when determining the electrical resistance of liquids? (2) Does Ohm's law also apply to decomposable conductors?[38] And connected with this: How can Clausius' postulate be proven that Ohm's law still holds for the smallest possible electromotive forces? (3) How does the electrical resistance of decomposable conductors depend on their concentration?[39]

Clausius explicitly pointed out in his paper about free ions from 1858, discussed above, that "*The smallest possible force gives rise to a current accompanied by alternate decompositions and recombinations, and the intensity of this current increases in proportion to the force, according to Ohm's law.*" Since Clausius gave no experimental results for this "smallest possible force", and did not cite any sources in which this force was given,[40] we find this central argument vague and indefinite and a weak point in Clausius' chain of reasoning (see also footnote [32]).

However, Kohlrausch and Nippoldt took up this challenge and attempted to measure a critical value of this force. Unable to achieve a sufficiently low alternating current, they applied thermoelectric current instead, generated by a pair of Cu-Fe wires. And indeed, Kohlrausch and Nippoldt were able to spectacularly determine the lowest electromotive force at which the electrolytic decomposition of the zinc vitriol solution still occurred, i.e., they measured whether or not current and emf were in direct proportion.[41] Their results led them to conclude that *"… die Gültigkeit des Ohmschen Gesetzes … bis zu einer elektromotorischen Kraft von 0,00000233 oder 1/429000 Grove hierdurch als bewiesen ansehen kann*" *(… the validity of Ohm's law … down to an electromotive force of 0.00000233 or 1/429000 Grove can thus be regarded as proven*). This emf in Grove is equivalent to $4{,}2.10^{-6}$ V. Referring to the work of H. Buff from 1855,[110] Kohlrausch and Nippoldt deduced that this weak electrical force which decomposed their electrolyte was greater than the force of its chemical affinity. Thus they agreed with Clausius' theory, but their conclusion was based on factual data (p. 379). They ended the paper with the nonetheless qualifying remark

> Die Prüfung des Ohmschen Gesetzes für Elektrolyte auf noch kleinere Kräfte als die obigen auszudehnen, darf weder als überflüssig noch als unmöglich bezeichnet werden. (To extend the test of Ohm's law for electrolytes to even smaller forces than the above may be said to be neither superfluous nor impossible.)

The first and third questions posed above related to one of the main sources of errors in measuring the electrical resistance and conductivity of liquids, namely the polarization of the electrodes. Two options were known to minimize polarization. To use alternating instead of direct current; and to measure with amalgamated zinc electrodes, that were not polarized. Kohlrausch and Nippoldt opted for alternating current, which they generated in their laboratory using a rather elaborate device they built themselves.[42] They mounted, in addition, large-area platinum electrodes to reduce the current density there.

Sulfuric acid as the test substance was diluted in water at different percentages[106, 107] and the resistances were determined.[43] Their reciprocals, the "relative conductivities", are plotted in Figure 4. They were in accordance with earlier contributions by other authors, but were more accurate.

The curve of the relative conductivity of the solution vs. the specific weight of the sulfuric acid diluted in steps of ten (percentage of acid) increased at low concentrations, but then reached a maximum at 1.233 specific weight which equals 31.5 weight % of sulphuric acid hydrate (the "hydrate theory" is addressed in the following Part 4 of our historical reviews).

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[38] Kohlrausch and Nippoldt called here electrolyte solutions, i.e., 2nd class conductors, as opposed to metals, "decomposable conductors".

[39] Kohlrausch, of course, was not the first to research the conductivity or resistance of electrolyte solutions. Such earlier investigations had been discussed and compared in G. Wiedemann`s "Galvanismus"(ref. [108], pp. 191-208). Wiedemann reported on A. de la Rive (1830); G. T. Fechner (1831), see the Chapter "Leap in Time" above; C. S. M. Pouillet (1837); M. E. Lenz (1838); F. C. Henrici (1845) W. G. Hankel (1846); E. Becquerel (1846); E. N. Horsford (1847); E. Becker (1850, 1851); G. Wiedemann (1856); A. Saweljew (1856); W. Schmidt (1859). None of these researchers, however, has systematically studied this topic for about four decades and none of them has contributed with such fundamental findings. In this respect, the justification that Kohlrausch has coined the topic seems appropriate.

[40] In a letter to the Chairman of the Deutschen Bunsengesellschaft, W. Nernst, from May 24, 1908, Kohlrausch himself wrote about Clausius' argumentation and his and Nippolt´s measurement: "*Nach den von Clausius entwickelten Vorstellungen dürfte dies wohl als wahrscheinlich gelten, war jedoch niemals an der Erfahrung geprüft worden. Diese Lücke wurde ausgefüllt.*" (*According to the ideas developed by Clausius, this is very likely, but had never been tested on experience. This gap was filled.*).[109], p. 385. Note that Kohlrausch insisted on a test.

[41] The solution of zinc vitriol (i.e., zinc sulphate) electrolyzed by amalgamated zinc electrodes to exclude polarization contained 16.6 g of the salt in 100 g solution. This solution was filled into a tube with 2400 mm^2 cross-sectional area and had a length of 83 mm. The current was measured with Nobili's astatic galvanometer.

[42] In their first electrochemical experiments, Kohlrausch and Nippoldt used a rather complicated apparatus to generate alternating current, which was later replaced by one that was much easier to handle. They produced equal but oppositely directed currents of short duration and rapid succession by induction with a rotating magnet using large-area platinum electrodes (later, a small induction coil was used). The number of revolutions of the magnet was determined by means of a siren fixed in the axis of the magnet and its pitch was compared with a set of organ pipes. The induction currents were observed with a sensitive Weber bifilar dynamometer, which was later replaced by a Wheatstone bridge.

[43] The resistance was related to that of mercury.

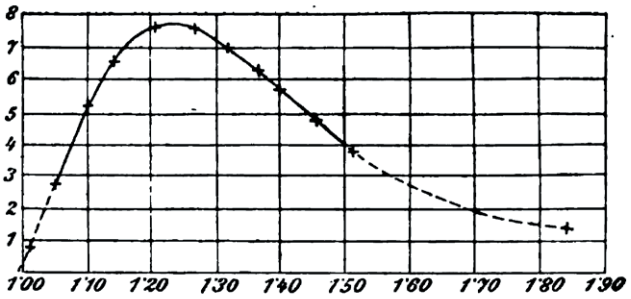

Figure 4. "Relative Leitfähigkeit" (relative conductivity) vs. specific weight (also density) of dilute sulphuric acid. The resistance was measured with alternating current as described in the main text. Abscissa: specific weights of the acid solution at 18.5°C from 1.0504 to 1.5025, corresponding to a content of the acid from 8.3 to 60.3%. The curve depicts the "Leitungsvermögen" at 22°C. From ref. [107], p. 386.

Kohlrausch´s finding that the conductivity depends on the concentration of the dissolved electrolyte initiated one of his major research activities. He carried out analogue measurements, also with alternating current,[44] and published the results in 1875 with Otto Grotrian,[111, 112] and in 1876 in a paper with a similar content, but with an improved experimental set-up.[113] In the same year he published the preliminary version of the *law of independent ion migration*,[114] the subject of the following chapter.

The Law of Independent Ion Migration: 1st Kohlrausch Law

Around the 1850s several authors, to name Wilhelm Hankel,[115] Gustav Wiedemann[39, 47] and Wilhelm Beetz[116] investigated the relationship between the electrical resistance of electrolyte solutions and their viscosity.[45] In 1856 Wiedemann came to the preliminary conclusion that under the conditions of his experiments (ref. [47], p.229) " *… würde … der Leitungswiderstand der Zähigkeit der Flüssigkeiten direct … proportional seyn.* (*…would … the resistivity be directly proportional to the viscosity of the liquids.*)[46]

The German physicist Georg Quincke[47] modeled in 1871 the resistance to the motion of "*Theilmolecüle*" (*partial molecules*), the ionic constituents of a "*Gesammtmolecül*" (*complete molecule*) in a thin thread of a liquid solution and brought into play not only the viscosity of the liquid but also the mutual attraction and repulsion of the ions.[123] He argued that this electrical interaction is compensated by the corresponding effect of the neighboring counterion and is therefore irrelevant, and concluded that

> Die specifisches Leitungsfähigkeit der gesamten Flüssigkeit ist gleich der Summe der partiellen specifischen Leitungsfähigkeiten der einzelnen Bestandtheile. (The specific conductivity of the entire liquid is equal to the sum of the partial specific conductivities of the individual components.)

This reads in eq. (26) on p. 16 that $\lambda = \lambda_1+\lambda_2+\ldots\lambda_r$. Here, λ is the specific conductivity of the solution, and λ_i are those of the individual ionic constituents, the *partial molecules*, with *i* ranging from 1 to *r*.[48]

In the second half of the 1870s Otto Grotrian found a striking connection between electric resistance and mechanic frictional resistance.[124, 125] Rudolf von Lenz, in the same years, investigated a relationship between the resistance of haloïd salt solutions and their density (not of their viscosity).[126, 127] All researches agreed that the liquid of the solution decelerates the motion of the ions and thus increases the resistance of the current. By the way, this is a prerequisite for a constant migration velocity of an ion driven by a constant force in the theory of electric lines of action of Michael Faraday (see Part 2, Chapter 3.1.6.).

This point of view was shared by Kohlrausch, who supposed that the source of the frictional resistance and thus the influence on the migration velocity of the ions in dilute aqueous solutions can only come from the water molecules due to their large excess over all other constituents. Taking into account this connection between electrical force and mechanical resistance in ion transport, Kohlrausch came up with the key idea that

[44] The salt content in the solutions was between ca. 3% and 30% (w/w). It seems perhaps superfluous to nearly always indicate the concentrations of the electrolytes in the respective measurements. However, this is not the case, since they play a central role in the migration properties of the ions.

[45] The device to measure the viscosity was based on the findings of the German hydraulics construction engineer Gotthilf Ludwig Hagen[117] and the French physiologist and physicist Jean Léonard Marie Poiseuille.[118][119][120] The device resembled the nowadays used standard U-tube viscometer.

[46] See also refs. [39], pp. 169, 170; [108], pp. 421-426; [121], pp. 632-634, and [122]

[47] Georg Hermann Quincke (1834, Frankfurt/Oder – 1924, Heidelberg) studied physics, chemistry and mathematics, and presented his doctoral thesis on "*Kapillarerscheinungen bei Quecksilber*" (*Capillary phenomena with mercury*) at the Humboldt-University in Berlin in1858. In 1875 he became successor of Gustav Kirchhoff at the University of Heidelberg, where he retired in 1907. Interesting for the present subject are his scientific activities in capillarity, in the electric properties of colloidal particles and in electroosmosis (we will come back to these important contributions in a later review).

[48] Note that Quincke used Clausius´ phraseology, and that this equation has some similarity with Kohlrausch´s law of independent ion migration.

the extent of this mutual influence is most likely different for different ions, which would lead to their unequal migration velocities. He communicated his hypothesis in 1876 – the preliminary version of the law of independent ion migration – and confidently stated (ref. [114], p. 215)[49]

> Ist nun die Lösung sehr verdünnt, so wird diese Reibung vorwiegend an den Wassertheilchen stattfinden. Demnach wird man weiter zu schließen versucht sein – *und dies ist ein Schluß, der meines Wissens noch nicht gezogen worden ist* – daß jedem elektrochemischen Elemente (z. B. dem Wasserstoff, Chlor oder auch einem Radicale wie NO_3) als solchem ein bestimmter Widerstand in verdünnter wässeriger Lösung zukommt, gleichgültig, aus welcher Verbindung es elektrolysirt wird. (If the solution is very dilute, this friction will mainly take place on the water particles. Accordingly, one will be tempted to conclude further – *and this is a conclusion which, to my knowledge, has not yet been drawn* – that every electrochemical element (e.g., hydrogen, chlorine or even a radical like NO_3) as such has a certain resistance in dilute aqueous solution, regardless of the compound from which it is electrolyzed.)

He argued, on the one hand, that the current is related to the sum of the velocities ($\nu_-+\nu_+$) of the two oppositely charged ions.[50] The current is, according to Ohm's law, also in inverse proportion to the resistance, that is to say, it is proportional to conductivity Λ. Hence the conductivity is proportional to ($\nu_-+\nu_+$).[51] He pointed out that, on the other hand, Hittorf´s transference number expresses the ratio of the velocity of an ion to the sum of the velocities of both ions, e.g., for the anion by $\tau_-=\nu_-/(\nu_-+\nu_+)$. For two electrochemically equal electrolytes, (1) and (2), with a common anion and with cation velocities of $\nu_+^{(1)}$ and $\nu_+^{(2)}$, respectively, he set $\Lambda^{(1)}/\Lambda^{(2)}=(\nu_-+\nu_+)(\nu_-^{(1)}+\nu_+^{(2)})$, expanded the equation by ν_-, and obtained the relationship $\Lambda^{(1)}/\Lambda^{(2)}=\tau_-^{(2)}/\tau_-^{(1)}$. The corroboration of his hypothesis required that the ratio of the conductivities of the solutions of two electrolytes with a common ion had to be equal to the inverse ratio of the transference numbers of the common ion (it was already noted above that the transference number of an ion in an electrolyte depends on its counterion).

Kohlrausch scrutinized his hypothesis on the basis of transference numbers of about a dozen electrolytes from monobasic acids[52] (salts of alkalis and earth alkalis as chlorides, bromides, iodides, nitrates, perchlorates and acetates), and actually found a good agreement with his theoretical prediction. Thus he concluded on p. 219

> Die Annahme von der unabhängigen Beweglichkeit der Ionen läßt sich zweitens durch die Überführungszahlen allein prüfen und hierdurch auch an Körpern, deren Leitungsvermögen noch nicht bekannt ist, bestätigen oder widerlegen. (Secondly, the assumption of the independent migration of the ions can be proved by the transfer numbers alone and thus confirmed or disproved on bodies whose conductivity is not yet known.)

To this end, Kohlrausch took four compounds, which were composed of two pairs of ions, and related their resulting eight transference numbers to one another. In fact, he was able to calculate "Beweglichkeiten" (verbatim "mobilities"), the velocities relative to that of hydrogen. This hypothesis must not be confused with the final *law of independent ion migration* Kohlrausch published three years later.[130]

At the end of his 1876 paper (on p. 222) he came nevertheless to the conclusion that further experimental investigations were necessary to decide whether this aforementioned statement applies also in general or not. He began to examine systematically and with exceptional accuracy numerous aspects of the influence of various

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[49] In this early paper he took values for electrolyte concentrations in the low weight percent range.

[50] In the following discussion electrolytes with two single-charged ions are considered for the sake of simplicity (for other electrolytes the corresponding equations have to be extended by the numbers of ions, ν, and the charge numbers, z. Since electrolytes with $\nu_-=\nu_+$ and $z_+=|z_-|=1$ are regarded, the molar and the equivalent concentrations and the respective conductivities are the same.

[51] We point out that at the time of Kohlrausch there was no standardized terminology, nor were the symbols of the physical and chemical quantities unified. Kohlrausch, for example, used the terms_"Leitungsvermögen" or "Leitfähigkeit" of the solution, which is "conductibility" or "conductivity" in English, and ascribed it in some papers by symbol *k*, in others by Λ. More misleading is Kohlrausch´s usage of the term "Beweglichkeit", with symbols l_+ and l_-, in other papers *u* and *v*, which is translated into English as "*mobility*" (a point that has been mentioned in the English translation of Kohlrausch´s paper from 1876[114] in Harper's Scientific Memoirs from 1899 (ref. [128], footnote at p. 89). In Kohlrausch's paper from 1876 "Beweglichkeit" is a dimensionless number, which is the velocity of migration of an ion related to that of hydrogen. The latter has the arbitrarily chosen value of 1. Kohlrausch initially formulated in his hypothesis of independent ion migration that the conductivity is the sum of the "Beweglichkeiten" of the ions, which he expressed by $\Lambda=(l_+ + l_-)$ or by $k=(u+v)$ (comp. with the different notations given in the main text). In later papers, Kohlrausch used the term "Wanderung" (Engl. migration) instead. Somewhat problematic for the reader of Kohlrausch's original works may be that he often changed terms during the four decades of his scientific activity. This is understandable, because, as we mentioned above, there was no standardized nomenclature. However, since this review is not intended to be a textbook of physical chemistry, we usually adopt the terms as Kohlrausch used it in the respective paper, unless they are completely misleading. In the cases where it could lead to confusion, we will use the modern terminology and symbols.

[52] It is hardly known that acids with 1, 2 and 3 H^+ ions and bases with corresponding OH^- ions were called mono-, di- and triatomic until 1853, when Faustin-J. (also Faustino) Malaguti (1802 – 1878) proposed in his "*Leçons élémentaires de Chimie*" (ref. [129], p. 331) to call them mono-, di- and tribasic acids and mono-, di- and triacidic bases, respectively. It is still modern nomenclature.

experimental variables on the conductivity of electrolyte solutions till around 1910 (that is, please note, still in the *Long Nineteenth Century*). During this time period, he published almost 145 papers on this subject.

But first he continued work on his above-mentioned hypothesis for the next three years and published his results in 1879 on 120 pages of an ample paper that consisted of three parts.[130, 131] In theoretical Part III[130] he derived in detail the *law of independent ion migration*, which is also named 1st Kohlrausch law. In § 15 of this Part III he defined the quantity *m*, which he called the molecular content, as the number of electrochemical molecules per unit volume.[53] He defined the "molecular Leitungsvermögen" (the molecular, later also molar conductivity), *k*/*m*, of the electrolyte in aqueous solution as conductivity, *k*, related to the molecular content, *m*. This quantity will be used by him in his future papers. Then, in § 18, he reported the remarkable finding of the similar difference in the molecular conductivities of two single-charged salts with a common ion. To give some examples: the difference for the pair *K* and *Li* is 274 for the chlorides, and 272 for the iodides; it is 160 for *K* and *Na* as chlorides, and 185 for their iodides; for the chlorides of *K* and NH_4 it is 21, and 14 for their iodides (all in the measure he used). These results encouraged him to formulate the final version of the *law of independent ion migration* in § 21, p. 168.[130] This derivation, expressed in modern notation, begins with the fact that the molar conductivity Λ of an electrolyte solution is the sum of the molar ion conductivities, λ_+ and λ_-, of the two ions, i.e., $\Lambda=(\lambda_++\lambda_-)$.[54] To obtain the conductivity of the individual ions, Kohlrausch combined Λ, as indicated above, with the transference number, τ, what led to the quested ion conductivity, e.g., for the anion as $\lambda_-=\tau_-\Lambda$, in which both, Λ and τ_-, are experimentally determinable measurands. Analogously, the cation conductivity is $\lambda_+=(1-\tau_-)$ or $\tau_+\Lambda$, because $(\tau_++\tau_-)=1$.

On p. 168, ref. [130] Kohlrausch formulated this *law of independent ion migration* (reproduced verbatim; note the almost identical wording of his 1876 hypothesis, that we quoted above, but which was, in contrast, formulated in subjunctive) as

> Hiernach muß also jedem elektrochemischen Elemente – z.B. dem H, K, Ag, …, Cl, J, NO_3,… – in verdünnter wäßriger Lösung ein ganz bestimmter Widerstand zukommen, gleichgültig aus welchem Elektrolyte der Bestandteil abgeschieden wird. Aus diesen Widerständen, welche für jedes Element ein für allemal bestimmbar sein müssen, wird sich das Leitungsvermögen jeder (verdünnten) Lösung berechnen lassen. (According to this, every electrochemical element – for example H, K, Ag, …,. Cl, J, NO_3,… – must feature a very definite resistance in dilute aqueous solution, regardless of which electrolyte the component is deposited from. From these resistances, which must be determinable for every element once and for all, the conductivity of each (dilute) solution will be calculable.)

He tested the validity of this theory by calculating the equivalent conductivity of the salts[55] by summarizing the tabulated conductivities of their ions (he completed his data with those from Robert von Lenz.)[126, 136],[56] Even after taking into account electrolytes with double-

[53] We have already mentioned that, tedious for the current reader, Kohlrausch often changed the nomenclature and the phraseology during the long time of his researches. In this § 15 on p. 146 of ref. [130] he defined *m* as "*Die Anzahl der …. electrochemischen Molecüle, welche in der Volumeneinheit enthalten sind, werde ich kurz die Molecülzahl oder den Moleculargehalt der Lösung nennen und durch m bezeichnen.*" (*The number of …. electrochemical molecules contained in the unit volume, I will briefly call the molecule number or the molecular content of the solution and denote it by m*) It is a relative quantity, which is calculated "*Aus dem Procentgehalt und dem specifischen Gewicht bei 18° berechnet man die in 1 ccm Lösung enthaltene Milligrammzahl des Electrolytes und theilt letztere Zahl durch das electrochemische Moleculargewicht der Substanz.*" (*From the content in percent and the specific weight at 18°, one calculates the milligrams of the electrolyte contained in 1 ccm of solution and divides the latter number by the electrochemical molecular weight of the substance*). It clarifies the matter by Kohlrausch´s statement on pp. 172-173 in ref. [132] "*Die untersuchten Flüssigkeiten sind bezeichnet nach ihrem Gehalte an „electrochemischen Molecülen" (Aequivalenten) in der Volumeneinheit. Der „Moleculargehalt" m bedeutet …. die in 1 L der Lösung enthaltene Menge in Grammen, getheilt durch das Aequivalentgewicht A des Körpers. m = 1 bedeutet also die bei der Titriranalyse sogenannte „Normallösung.*" (*The liquids examined are designated according to their content of "electrochemical molecules" (equivalents) in the unit of volume. The "molecular content" m means … the quantity in grams contained in 1 L of the solution, divided by the equivalent weight A of the compound. m = 1 therefore means the "normal solution" as it is known in titration analysis.*) Later Kohlrausch called *m* the equivalent concentration (what Faraday named electrochemical equivalent concentration).

[54] We shall discuss in the following section that, taking into account the results of his research on the concentration dependence of conductivities, the modern notation of this equation reads $\Lambda^0=(\lambda_+{}^0+\lambda_-{}^0)$; superscript 0 indicates limiting conditions, that is, when the concentrations approach zero; see also, e.g., refs. [133][134][135]

[55] The concentrations of the salts were between around 5 weight % and their solubility limit, which reached for some salts about 80 weight %.

[56] The little-known Baltic-Russian physicist Robert von Lenz (1833, St. Petersburg -1903), son of the German-Baltic physicist Heinrich Friedrich Emil Lenz (1804, Dorpat, now Tartu, Estonia – 1865, Rome) known for Lenz's law in electrodynamics, was professor of physical geography at the University of St. Petersburg from 1865 to 1899. He published a paper in 1877, read in 1876, about conductivities of haloïd salts. Lenz´s conclusions there resembled Kohlrausch´s law of independent ion migration.[126][127] In 1879 he published conductivities of aqueous alkali salt solutions in the concentration ranges from about 10^{-1} to 10^{-3} equ.L^{-1}.[136] Notably, Lenz was the first who systematically determined transference numbers, conductivities and diffusion coefficients of ions in mixed aqueous-ethanolic solutions up to ethanol concentrations of about 94% (v/v). He confirmed the proportionality of conductivity and diffusion rate,[137] which was later expressed quantitatively (for limiting conditions) by the Nernst-Einstein equation.

charged ions, Kohlrausch found that the calculated values were in satisfactory agreement with the measured ones, considering the limited reliability of the transference numbers.[57] Eventually, Kohlrausch concluded

> In diesen Beispielen sehen wir unsere Vermuthung, dass die Beweglichkeit eines Jons in verschiedenen Verbindungen die gleiche ist, mit grosser Annäherung bestätigt. (In these examples we see our conjecture confirmed with great approximation that the mobility of an ion is the same in different compounds.)

Kohlrausch´s finding that ions migrate in the electric potential independent of the counterions was a milestone for the further development of the theories of ion migration.

The Concentration Dependence of the Conductivity, and the Conductivity at Limiting Conditions

In the above chapter we have described Kohlrausch's first experiments in 1869 and 1870 on the conductivity of electrolyte solutions. This subject fascinated him so much that he introduced his next paper of July 1874 (the precursor of refs. [111, 112]) with the prophetic sentence: "*Diese in Gemeinschaft mit Hrn. Grotrian ausgeführte Arbeit soll den Anfang einer geordneten Experimental-Untersuchung über die Strom-Arbeit im Inneren der Elektrolyte bilden* " (*This work, carried out in collaboration with Mr. Grotrian, is to form the beginning of an orderly experimental investigation of the work of the current inside electrolytes*). This undertaking then actually extended over four decades. Following this stated intention, in 1875 and 1876 he investigated the resistance or conductivity of a large number of solutions of salts, bases, organic and inorganic acids and their dependence on the electrolyte content.[111-113, 144]

Their electrolyte concentrations ranged from full saturation down to a few percent by weight. Kohlrausch found a steady increase in conductivity with increasing concentration, with some electrolytes having a maximum at a particular concentration. For the discussion and comparison of the conductivities of the various electrolytes, he considered the indication of the concentration in percent by weight to be inappropriate. The conductivities of sulfuric acid and acetic acid, for example, could be determined at 100 %, while oxalic acid reached only 7 %, both (w/w). Note that also in 1876 he published the preliminary version of the law of independent ion migration (ref. [114], see previous section). These measurements were also carried out with solutions with electrolyte concentrations not lower than a few weight percent.[58]

The "Cubic-Root" Relation, the Precursor of the "Square-Root Law"

In his paper from 1885 "*Ueber das Leitungsvermögen einiger Electrolyte in äusserst verdünnter wässeriger Lösung*" (*On the conductivity of some electrolytes in extremely dilute aqueous solution*)[132] Kohlrausch presented his measured conductivities in the low electrolyte concentration range between 10^{-5} and 1.0 mol.L^{-1}. He completed the actual data with those for molecular concentrations larger than 1 mol.L^{-1} by his earlier ones and by those published by Long,[147] and obtained equivalent conductivities for concentrations up to nearly 10 mol.L^{-1}. Since it was not meaningful to plot the molar or equivalent concentration, m, in a linear scale over a range of about 6 orders of magnitude, he chose the cubic root of m as the abscissa.[59] The resulting k/m vs $m^{1/3}$ curves are shown in Figure 5.

Two shapes of these curves were clearly discriminable for Kohlrausch. He distinguished therefore two classes of electrolytes. Alkali salts of the type A^+B^- were typical representatives of the 1st class. For these electrolytes, the k/m values decreased only slightly by a few ten percent with increasing concentration in the low concentration range (Figure 5, upper scale). In contrast, 2nd class electrolytes like acetic acid and NH_3 exhibited a very low molar conductivity at high concentrations (pure acetic acid behaved as non-conductor). They remained at a low level when diluted over a wide concentration range, but rose steeply to values in the same range as those of the strong electrolytes when the solution became highly

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[57] Transference numbers with higher accuracy and with much less experimental effort than those by Hittorf´s method were later determined by the moving boundary method. This method will be discussed in a future paper together with electrophoretic separation methods in free solution. It was introduced in 1886 by Oliver Lodge,[138] and was further developed by W. C. D. Whetham,[139]. by O. Masson,[140] by A. Noyes,[141] by B. D. Steele and R. B. Denison,[142] and by others. It was theoretically clarified, in addition to other methods, in 1897 by Friedrich Kohlrausch with his "beharrliche Funktion" (the "persistent function", better known as "regulating function").[143] We will, however, not go into the details here.

[58] In 1868/69 also A. Paalzow measured the resistance of diluted salts and acids down to the low % range.[145][146] Rudolf Lenz reported conductivities of haloïd salts in 1877.[126][127] Later, experimental results and theoretical discussions about ion conductivities and their concentration dependence were published in 1880 by J. H. Long,[147] in 1884 by E. Bouty,[148][149][150] by Svante Arrhenius in his doctoral thesis,[151][152] which Kohlrausch provided with some pointed remarks, and by W. Ostwald.[153]

[59] Kohlrausch followed the suggestion of R. Lenz in 1878 to relate conductivity to the amount of electrolyte molecules in the solution.[136]

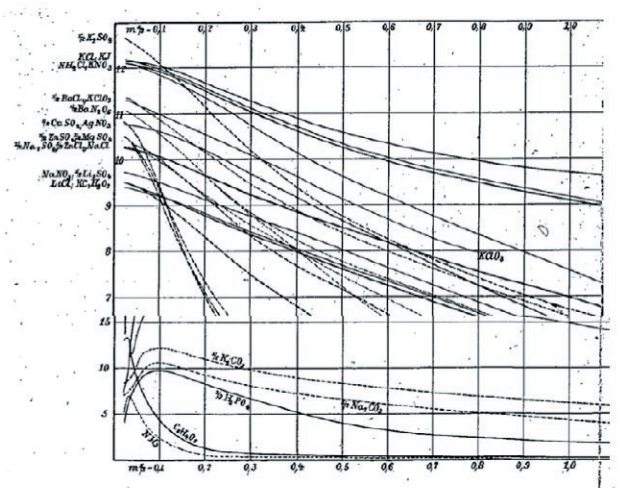

Figure 5. Equivalent conductivity ("specific molecular conductivity") as function of the cubic root, $m^{1/3}$, of the equivalent concentration, m. The figure shows the left sectors of the total plot, that between zero and 1 equ.L^{-1} concentrations. The curves were drawn exactly through the measured values. Upper scale, strong electrolytes, e.g. KCl, $KClO_3$, etc. Lower scale, concave two lowest curves: weak electrolytes acetic acid, NH_3. Modified; from ref. [132], Plate II, after p. 648.

Figure 6. Photograph of Friedrich Kohlrausch´s research group at the Physical Institute of the University in Würzburg in winter semester 1886/87, taken in February 1887. Standing from left, Adolf Heydweiller, Ewald Rasch, Svante Arrhenius (research visit in Würzburg in 1886/1887), Walther Nernst. Sitting from left, Wilhelm Kohlrausch, Friedrich Kohlrausch, Samuel Sheldon (postgraduate; 1887-1888). Source of photograph: Ernst H. Riesenfeld. Svante Arrhenius. Akademische Verlagsgesellschaft, Leipzig, 1931. Public domain.[154]

diluted (see the two lowest and concave curves in the bottom scale of Figure 5.)

Kohlrausch recognized from the curves that the molar (or equivalent) conductivities of both classes of electrolytes nevertheless reached a certain limit at concentrations approaching zero.[132] He expressed k/m as a function of m for dilute solutions of strong electrolytes by the equation $k/m=A-Bm^{1/3}$. This relationship stated that the equivalent conductivity is proportional to the cubic root of the concentration, and under limiting conditions it is equal to constant value A. For salts from single-charged ions, if only for these, the curves were approximately linear up to the concentration range of 1 mol.L^{-1} (see, for example, that for $KClO_3$ in the figure). Kohlrausch interpreted this dependence by the assumption that $m^{1/3}$ corresponds to the reciprocal mean distance between the electrolyte molecules. For the 2nd class electrolytes he had no coherent explanation. This cubic root equation, which applied to a relatively large range of concentration, preceded Kohlrausch´s "square-root" equation for extremely dilute solutions which will be discussed in the following.

After the investigations in 1885 Kohlrausch was aware of the insufficient accuracy of the data measured so far, as he assessed their quality as too low for the formulation of a sound and general theory, especially with regard of the values at very low concentrations. He therefore tried to identify and reduce the possible sources of errors. This was the goal of his comprehensive and elaborate studies over the next years.

In Figure 6 a photograph of Friedrich Kohlrausch´s group at the University in Würzburg in 1887 is shown. It was taken when Kohlrausch was visited by Svante Arrhenius, who published in this year his seminal dissociation theory *"Ueber die Dissociation der in Wasser gelösten Stoffe"* (*On the dissociation of substances dissolved in water*).[155],[60] Although Arrhenius' theory provided the explanation for the deviating dependence of the 2nd class electrolytes such as acetic acid, Kohlrausch was skeptical of it. He continued to focus on strong electrolytes at limiting conditions, preferably on those with single-charged ions.

In strict chronological order we had now to proceed with Arrhenius' dissociation theory from 1884.[151, 152]. However, we prefer to continue systematically with Kohlrausch's electrochemical work after 1887,[61] and will

[60] Arrhenius completed his doctoral thesis in 1884. It was published in two parts of "*Recherches sur la conductibilité galvanique des électrolytes*."[151][152] In 1887, his dissociation theory was published in the first volume of *Zeitschrift für physikalische Chemie, Stöchiometrie und Verwandtschaftslehre*,[155] founded by Wilhelm Ostwald and Jacobus Henricus van `t Hoff. In 1928 its title was changed to *Zeitschrift für physikalische Chemie.* Arrhenius' theory fundamentally changed the previous conception of the behavior of electrolytes in solutions, and was a decisive step in the development of modern physical chemistry. In 1903 he became the first Swedish Nobel laureate.

[61] It should be noted that in 1889 and 1890, due to adverse circumstances, Kohlrausch did not publish a single paper.

return to Arrhenius' theory and its pioneering consequences in the following, separate Part 4 of our series.

The "Square-Root" Law: 2nd Kohlrausch Law

In order to achieve the proverbial high accuracy of his experimental results, for which Kohlrausch was famous, he examined step by step the effects of possible causes of errors. He tried to reduce the total systematic error at conductivity of solution with molecular concentration of 0.0001 which required errors in conductivity one magnitude lower in each individual step at molecular concentrations of 0.00001. We list a part of the subjects of these studies in condensed form in footnote [62], along with the articles in which they were published. It turned out that two sources of errors were most problematic. One was the well-known polarization of the electrodes, a problem Kohlrausch solved, as he had done earlier, by using alternating rather than direct current. He used, in addition, an improved apparatus, the Wheatstone bridge, for the measurement of the resistance.[63]

The second problem was that the water used as a solvent had to be of very high purity. Kohlrausch's coworker Adolf Heydweiller countered this problem in 1894 with cumbersome and time-consuming purification processes.[158, 172]

In their paper from 1900, Kohlrausch and his coworker E. M Maltby published very accurate conductivities of alkali halides and nitrates in highly dilute solutions in a comprehensive report which covered eighty printed pages and summarized the results of their elaborate investigations.[133] Taking the data at lowest m between 1.10^{-5} and 4.10^{-5} equ.L^{-1} (out of a set over a range of up to 1 equ.L^{-1}) Kohlrausch expressed the dependence of the equivalent conductivity, here symbolized by Λ, on m by the equation Λ_0-Λ=$Pm^{1/2}$; Λ_0 and P are electrolyte-specific constants.[133],64. He called this equation "Quadratwurzel Gesetz" (literally "square root law", better known in English literature as 2nd Kohlrausch law).[65] The equation signifies the linear dependence of the equivalent conductivity of the electrolyte on the square root of its equivalent concentration, and again applied especially for strong electrolytes with single-charged ions. The intercept Λ_0 represents the limiting equivalent conductivity of the electrolyte at concentrations $m\rightarrow 0$. Constant P, the slope of the line, depends mainly on the stoichiometry of the respective electrolyte. Note that the concentration to the power of ⅓ in the equation in his paper from 1885[132] is substituted here by the power of ½, because the former applied for a much larger concentration range. The decisive factor for the better agreement of the $m^{1/2}$ – relationship was not the lower measurable concentration of the electrolytes, it was the higher accuracy of the conductivity data at these extremely low concentrations. We illustrate this excellent relationship in Figure 7,[135] when Kohlrausch had available a larger number of more accurate data.[66]

The following paper of 1907 is a kind of résumé of Kohlrausch´s electrochemical contributions; in it he reconfirmed his earlier works. He subjected his theories of independent ion migration and those of conductance and mobility at limiting conditions to his own criticism. Moreover, he reaffirmed the validity of his square root law from 1900[133] not without emphasizing that this law applies only to salts of single-charged ions, and to concentrations not higher than a few 10^{-5} mol.L^{-1}. For higher concentrations this dependence is represented – as described in the above chapter – by the cubic root equation.[133] Kohlrausch also mentioned the alternative equations by Max Rudolphi[173] and J. H. van ´t Hoff,[174] which read in his version $(\Lambda_0-\Lambda)/\Lambda^p=C\eta^{1/2}$. Exponent p was taken as 2 by the former, and 3/2 by the latter author; η is here the equivalent concentration. Kohlrausch pointed out that these equations, however, apply only to individual cases. In 1908 he published three

[62] (▪) Influence of solubility of glass ware in contact with the solutions on the conductivity; ascertaining threshold level of conductivity of pure water used as solvent.[156][157][158] (▪) Corrections taking into account temperature coefficients of water and of solutions, comparison with previous measurements.[132][159] (▪) Estimation of deviations of electrolyte concentrations upon contraction by mixing of solutions and by evaporation of solvent.[160] (▪) Measurement of resistance of electrolyte solutions with direct and alternating current.[161][162][163][164] (▪) Density measurements of dilute electrolyte solutions.[160][165] (▪) Determining and improving the properties of rheostat, capacitor and Wheatstone bridge. [166]. (▪) Specification and improvement of quality of resistor cells and electric wiring.[167] (▪) Investing effect of polarization of platinized electrodes in measuring cell.[168][169] (▪) Calibration of thermometer to 0.01° instead of common 0.1° (§ 15) and of the volumina of graduated measuring glassware (§ 16, volumetric flasks; § 18, pipets) in ref. [133]

[63] The principle of the device called Wheatstone bridge was initially described by the British mathematician and physicist Samuel Hunter Christie (1784, London – 1865, Twickenham, London) and reported in 1833 in his Bakerian Lecture "*Experimental Determination of the Laws of Magneto-electric Induction in different masses of the same Metal, and of its Intensity in different Metals.*"[170] It was not noted until Charles Wheatstone presented it ten years later, mind you, as Christie's invention, in his Bakerian lecture "*An Account of Several New Instruments and Processes for Determining the Constants of a Voltaic Circuit.*"[171]

[64] In this concentration range the conductivity of water (purified by up to 40 to 50 distillations under vacuum from a quartz apparatus)[158] was about one order of magnitude lower than those of the highest diluted electrolyte solutions.

[65] Remember that the 1st Kohlrausch law is the law of independent ion migration.

[66] The data were taken from sources Kohlrausch cited in footnote 3, p. 336, ref. [135]

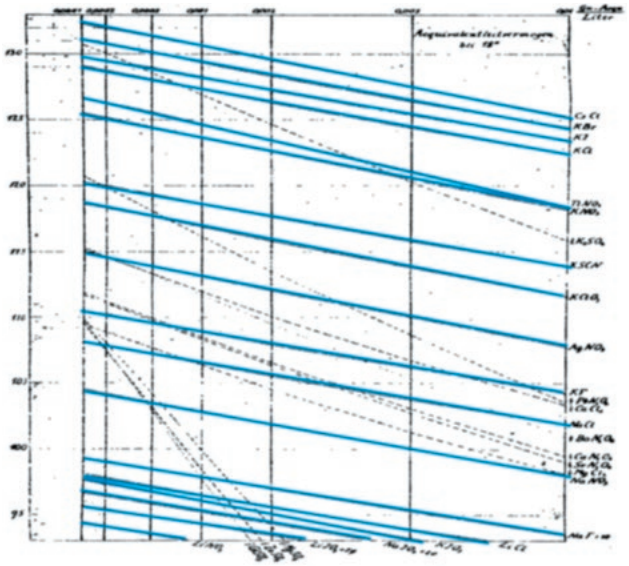

Figure 7. Dependence of the equivalent conductivity on the square root of the equivalent concentration according to the 2nd Kohlrausch law, the square-root law (termed "Quadratwurzel Gesetz" by Kohlrausch) for 1st class electrolytes. The straight lines of electrolytes with the same stoichiometry run parallel, most pronouncedly for salts with 1:1 single-charged ions. They are highlighted in blue. Since the letters in the illustrations of that time were mostly very small and hardly legible, we give the formulas of these salts in the order from top to bottom as follows: *CsCl*; *KBr*; *KCl*; KNO_3; *KSCN*; $KClO_3$; $AgNO_3$; *NaCl*; *NaF*; *LiCl*; KJO_3; $NaJO_3$; $LiJO_3$; $LiNO_3$. Ordinate, equ. conc.; abscissa, equ. conductivity. Modified, from [135].

more papers on electrochemistry, mainly on temperature effects of electrolyte solutions, and on January 4, 1910 he submitted a paper on "*Practical Rules for Number Corrections, Namely for the Transition to Other Atomic Weights*".[175] He died on January 17, 1910.

Migration Velocity and Mobility of ions

The question about the absolute migration velocities of the ions, not only about their relative values, has been systematically treated by Kohlrausch in several papers. [114, 130, 159, 176] In 1893 he had available improved values of the conductivity in dilute solutions, a valuable addition of Hittorf´s transference numbers, more reliable values for the electrochemical equivalents and the absolute resistance of mercury. Therefore, he was able to calculate more accurate data "*Ueber die Geschwindigkeit elektrolytischer Ionen*" (*On the velocity of electrolytic ions*).[159] He limited the following calculation to strong electrolytes, from which he could be convinced that the molecules completely decompose to ions (we recall that Arrhenius dissociation theory was published as journal article in 1887). We place his derivation into footnote [67] and add only the result after transformation into modern system of units and terminology.

Kohlrausch derived an equation for the migration velocities at unit field strength, that is to say, at 1 $V.cm^{-1}$, which he called "Beweglichkeiten" (mobilities), and which are, after multiplied by the according factor, the migration velocities in $cm.s^{-1}$. By transformation this mobility reads $\mu_i=\lambda_i/F$, at limiting conditions $\mu_i^0=\lambda_i^0/F$, and is today also called mobility of ion, *i*. Here λ_i is the molar ion conductivity, and *F* the Faraday constant. The velocity *v* in $cm.s^{-1}$ at field strength *E* is accordingly $v_i= \mu_i.E$.[68]

Ion mobilities μ_i are of decisive importance in all variants of capillary electrophoresis in free solution. They are ion specific parameters that are independent of the potential. They depend on temperature, on electrolyte concentration (more precisely, on ion strength, what was unknown at that time) and on the solvent.[69]

Since the migration velocity can be derived from the known ion conductivity and the chosen field strength, the question posed in this Part 3 about its magnitude can be regarded as answered.

However, the reason for its dependence on experimental variables, especially on their concentration, was still unknown at that time.

The reader will probably note that we have not yet addressed the main question of whether or not electrophoresis experiments in free solution were performed in devices of capillary format in the years under consideration. For this reason, we conclude the present essay by describing the first use of capillaries in electrophoresis and additionally mention that all experiments cited below were performed with direct current.

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[67] Kohlrausch derived the velocities for single-charged 1:1 electrolytes, that is, the molar and equivalent concentrations are the same. He considered a solution with molar concentration *m* of the electrolyte, which has the conductivity, *k*, and the "molecular conductivity" *k*/*m*, which is the sum of the molar conductivities of anions and cations. He derived the migration velocities of the ions at a potential difference of 1 $V.cm^{-1}$. He first changed conductivity *k* from its relationship to mercury to Ohm. He calculated the current in Amp. at 1 $V.cm^{-1}$ in 1 cm^3 of electrolyte solution with *m* $mol.L^{-1}$ concentration, and derived the number of moles electrolyzed by this current. This quantity is released at the electrodes at a distance of 1 cm. From this he derived the sum of the mean velocities of cations and anions and obtained those of the two ion species using Hittorf´s transference numbers. The resulting equation expressed the ion migration velocity in $cm.s^{-1}$, mind you, at a potential difference of 1 $V.cm^{-1}$, as a function of the molar ion conductivity. Readers who are interested in the specific numerical data in this derivation are referred to pp. 402-403 in ref. [159]

[68] In modern notation the migration velocity *v* is in $cm.s^{-1}$, the mobility μ in $cm^2.V^{-1}.s^{-1}$ or $S.cm^2.A^{-1}.s^{-1}$, and the molar ion conductivity in $S.cm^2.mol^{-1}$.

[69] Other researchers determined migration velocities of ions in the time period under consideration as well by the moving boundary method. We mentioned them in footnote[57].

THE FIRST CAPILLARY ELECTROPHORESES OF IONS

At the outset of this particular chapter in the history of electrophoresis, it should be made clear again that electrophoresis is not limited to the separation process as which it is generally regarded today, as already stated in footnote [1] in the introduction to this article. The history of capillary electrophoresis must therefore be considered from this point of view. To maintain continuity with the preceding narrative, we follow up the previous section with Kohlrausch's research around 1900 and address the topic in reverse chronological order.

1895. F. Kohlrausch and A. Heydweiller

In the course of their investigations on pure water in 1894[158] Kohlrausch and his coworker Adolf Heydweiller observed that after applying direct current the electrical resistance of water decreased rapidly. Changes in resistance were also observed in normally distilled and in highly distilled water and in some dilute aqueous salt solutions. Switching the current off and on resulted in some cases first in a decrease, after some time in an increase of the conductivity; in other cases, the reverse sequence was observed. One can follow their explanations of the reasons for these effects[70] in their paper "*Ueber Widerstandsänderung von Lösungen durch constante electrische Ströme*" (*On the change of resistance of solutions at constant electric currents*)[163] published in 1895. But this is of minor interest here.

Much more relevant to the issue at hand, they conducted their experiments in narrow open tubes of capillary dimensions because the zone boundaries described in footnote [70] were much sharper there than in tubes of larger diameters. They therefore used U-shaped capillaries with lengths of a few cm and inner cross-sectional areas of 1 mm^2 and 0.6 mm^2, that is, with radii of 560 and 440 µm. Kohlrausch and Heydweiller thus performed capillary electrophoresis – mind you – of ions. But they were far from being the first to introduce this method.

[70] They related it, for example, to the reaction of the solutions at the electrodes and the migration of boundaries of H^+ or OH^- formed by electrolysis from the one to the other electrode. Such effects were called by G. Wiedemann "*Ausbreitung der freien Säure vom positiven Pol aus*" (*Propagation of the free acid from the positive pole*), ref. [39], p. 167, and by H. Buff "*Ausbreitung der Säure gegen den negative Pol* " (*Propagation of the acid towards the negative pole*), ref. [177], p. 171. When both boundaries meet one another, neutralization takes place, water is formed, and the conductivity of the solution decreases. Kohlrausch and Heydweiller could visualize the motion of the boundaries by adding acid/base indicators.

1889. W. Ostwald and W. Nernst

We recall that Clausius' theory of free ions from 1858 was proved by Kohlrausch's 1869 experiment using thermoelectricity[107] as described in the chapter above. In 1888, Wilhelm Ostwald had theorized that free ions must be present in an electrolyte solution, otherwise the principles of electricity would be violated.[178] In 1889, together with Walter Nernst, he caught up with the experimental confirmation of his theoretical paper and described it in "*Ueber freie Ionen*" (*On Free Ions*).[179]

Ostwald and Nernst used an apparatus similar to Lippmann's capillary electrometer.[71] They modified the simplest version of this instrument, which is depicted in Fig. 3 on p. 503 of ref. [180]. In short, they took a tube of several tens of centimeters in length and reduced its lumen at one end to a capillary of $3{,}7.10^{-3}$ cm inner radius. They fixed the tube vertically, poured mercury into it, and immersed the tip of the capillary in dilute sulfuric acid. Then they sucked the mercury together with the acid into the middle of the capillary length. A platinum wire served for connection with the mercury. Next, they took a glass flask, covered with tin foil and filled with dilute sulfuric acid. This solution was connected to the acidic solution in the capillary via a wet thread. The mercury was grounded, and the positive pole of a small electrifying machine was connected to the outer tin foil of the flask. As soon as the machine was set in motion, the mercury meniscus promptly raised, indicating a potential difference between the two electrodes.

Remarkably, gas bubbles were formed, dividing the mercury in the capillary at several points. The authors explained this effect as follows. When the tin foil of the flask becomes positively charged, the negative sulfate ions are attracted, while the positive hydrogen ions are repelled. The latter travel through the wetted threat to the solution in the capillary and then through the platinum wire from the mercury to ground. The hydrogen transfers its electricity to the mercury in the capillary and appears as hydrogen gas.

[71] Lippmann's capillary electrometer enabled the measurement of very small numbers of electrical charges, and of potential differences down to a few tens of µV. Its main part consisted of a capillary half filled with liquid mercury in direct contact with a dilute solution of sulfuric acid. Both are connected with wires that serve as electrodes. Its principle is based on the relationship between the surface tension, the surface charge density of the mercury and the potential difference of the electrode points. Expressed by the Lippmann-Helmholtz equation, the surface tension of mercury is directly related to its surface charge density. A change in the potential difference leads to a change in the surface charge density, which in turn changes the surface tension. This causes the mercury meniscus in the capillary to rise or fall, which can be accurately measured using a microscope and, after calibration, gives the potential difference between the two electrode points.

Ostwald and Nernst argued that the motion is caused by influence, also known as electrostatic induction, and the formation of hydrogen on the mercury is clear evidence that decomposition has occurred. Since electrolysis does not occur without electrophoretic ion migration, the authors claimed that it was free ions that were moving. They also argued that only their explanation was consistent with the laws of thermodynamics (p.125 ff.). Taking this explanation as fact, Ostwald and Nernst performed capillary electrophoresis – again, mind you – of ions, six years before Kohlrausch and Heydweiller.

1865. W. Beetz

The German physicist Wilhelm Beetz[72] had already done research in this field in 1862, albeit with wider tubes, namely one with 13.4 mm i.d. and 297 mm length, the other with 6.2 mm i.d. and 207 mm length;[116] numbers rounded by us. His attention was drawn to the 1861 work of Edmond Becquerel on the conductivity of electrolyte solutions in capillaries,[181] which we will discuss in the next section. Beetz recognized that the conclusions in his work differed in part from Becquerel's. Doubting Becquerel's results, he decided to extend his measurements in 1865 from wide tubes to capillaries.[182] Thus, for comparison, he measured the resistances and calculated their reciprocal, the "Leitungsfähigkeiten" of electrolyte solutions in these capillaries.

For this purpose, Beetz constructed a device equipped with two Grove elements and determined the conductivities of zinc sulfate solutions with non-polarizing amalgamated zinc electrodes, and in six capillaries. The capillaries were between 77.7 and 161.2 mm long and had cross-sectional areas between 28.10^{-3} and 89.10^{-2} mm^2 (derived from the weights of the mercury-filled tubes), corresponding to inner diameters between 190 and 690 μ*m*; all numbers rounded by us. Beetz found that the resistances are directly proportional to the lengths of the capillaries, and are inversely proportional to the cross-sectional area of the capillaries.

1861. The Priority: Edmond Becquerel

In 1846 and 1847, the French physicist Edmond Becquerel[73] published the result of his investigations of the conductivity of electrolyte solutions that were filled in tubes with centimeter dimensions of inner diameter and length. In these works[184-186] he tested whether the relations of lengths and cross sections, as he had found in metal wires, also applies in electrolyte solutions.[74]

In the April 1861 issue of *Annales du conservatoire des arts et des métiers*, he published an essay with the designative title "*Études sur la conductibilité des liquides dans les tubes capillaires rhéostat destiné à la comparaison des grandes résistances*" (*Studies on the conductivity of liquids in capillary tubes; rheostat for the comparison of large resistances*".[181] In this paper he reported conductivities of liquids, but measured in capillaries of different inner diameters for comparison with resistances of metallic wires.

The device Edmond Becquerel constructed and used for his measurements in capillaries is presented in Figure 8. We describe in the following only the right one of the two glass tubes shown, because the other, similar measuring system had the same characteristics and was installed only for control and comparison. And it is described in more detail because – to the best of the author's knowledge – it was the first instrument ever used to perform capillary electrophoresis of ions in free solution.

The device consisted of a glass tube (*AB*) closed at the bottom and open at the top, with a diameter of 2 to 3 cm and a height of 50 to 60 cm. It was placed vertically in a larger glass vessel (*MN*) with 20 cm diameter and a height approximately equal to that of the tube. Water was filled into the large vessel to maintain the test solution, an aqueous solution of an electrolyte, at constant temperature. The tube was filled with the solution whose conductivity was to be determined. A capillary (*GH*) with a constant inner diameter and open on both ends, scaled in half-mm increments, was inserted in the axis of the tube and fixed there. The test liquid was filled into the tube and penetrated into the capillary, forming a narrow column of the electrolyte solution inside. Then a metal wire, (*cd*), made from copper, zinc, silver, or platinum, the type of which depended on the test solution,

[72] Wilhelm von Beetz (1822, Berlin – 1886, Munich) studied physics and chemistry in Berlin from 1840. From 1850 he was professor of physics at the Cadet Corps and at the Artillery and Engineering School in Berlin, from 1855 professor in Bern, from 1858 in Erlangen and from 1868 professor at the Technical University in Munich. His main field of research was electricity, especially topics of electrical conductivity of liquids.

[73] Edmond Becquerel, together with his father Antoine-César Becquerel, discovered in 1839 a variant of the photoelectric effect, later called the Becquerel effect. His main areas of research were phosphorescence of light and its chemical effects. He also investigated topics in optics and electricity. He is the father of Nobel Laureate (Antoine) Henri Becquerel. For a detailed biography of Alexandre-Edmond Becquerel (1820, Paris – 1891) see C. Blondel, ref. [183].

[74] It is remarkable that all authors discussed so far completely ignored Gustav Theodor Fechner´s comprehensive contribution about the validity of Ohm´s law for electrolyte solution, which he reported in his book "*Massbestimmungen über die galvanische Kette*".[69] He published the same findings already in 1831, see his conclusions formulated in points (i) and (ii) in Chapter "Leap in Time" above. Fechner, however, did not carry out his measurements in capillaries.

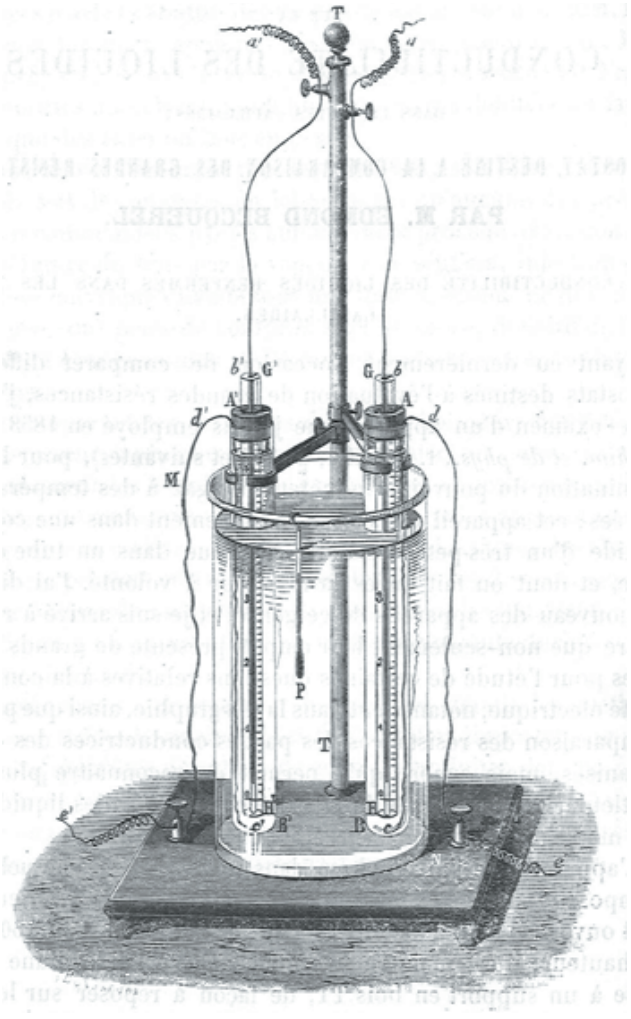

Figure 8. The rheostat constructed by Edmond Becquerel in 1861 for the first capillary electrophoresis of ions.[187] Explanations are included in the main text. Reproduced from Cnum – Conservatoire numérique des Arts et Métiers – http://cnum.cnam.fr with permission.

were inserted into the tube (*AB*). The end of the wire in the tube was placed near the bottom of the capillary (*GH*). The diameter of the other wire, (*ab*), which was inserted into the capillary from its upper opening, was very close to the inner diameter of the capillary, and acted like a piston. By pressing this wire, the length of the liquid column of the test solution in the capillary could be varied and its position could be measured on the calibrated scale with a magnifying glass.

The metal wires, (*cde*) and (*ab*), were connected to a Bunsen battery consisting of one to ten elements.[75] With this arrangement, the current was forced to flow through the test solution inside the capillary, the length of which could be varied by hand by the wire. The current was measured with an astatic galvanometer. By using capillaries with different cross sections the resistance and the conductivity of the electrolyte solution in dependence on the liquid length and its diameter could be determined. Five different capillaries were used with lumen radii of 929, 478, 238, 229 and 183 μ*m*. Polarization was minimized by using wires of the same metal as the cations of the test solutions.

Becquerel found that the resistance of such a liquid thread in a capillary is in direct proportion to its length, which is consistent with theory. However, he found that the product of resistance and the square of the inner diameter varied with decreasing diameter (of capillaries with the same length), and differed from that calculated for the given diameter (see Plate on p. 741).

On the one hand, Beetz agreed with Becquerel´s first conclusion. In contrast to Becquerel, however, Beetz found the resistances to be inversely proportional to the cross-sectional area of the capillaries, that is, the product of resistance and cross-sectional area is constant for capillaries of a given length. Beetz´s results were in agreement with the present theory, and also with Fechner´s early findings (see Chapter "Leap in Time"). Beetz's conclusions were plausible, and he had good reasons to explain the deviations from Becquerel's results, which he attributed to improper experimental conditions.

We note that Edmond Becquerel measured the conductivities, no matter whether right or not, and thus the migration properties of dissolved ions in an electric field, i.e., he actually performed electrophoresis. Even more, he was (as far as we know) the first to apply electrophoresis in capillaries – again, mind you – of ions.

It is noteworthy that the first capillary electrophoresis of colloidal or coarse-grained particles was performed around the same years, 1860 and then 1861, a fact we highlighted in the introduction to Part 1 of our series.[1] We will report in detail on this first capillary electrophoresis of colloidal particles in a later article. However, perhaps surprisingly, this will not be the work of either Nicolas Gautherot or Ferdinand Friedrich von Reuß, as they did not use capillaries in their discoveries.

SUMMARY

Around 1840, it was generally believed that the ions of strong electrolytes, like those of salts, migrate in solu-

[75] A Bunsen element was invented by Robert Bunsen in 1841.[188][189] It is an electrochemical zinc-carbon cell with zinc as anode in dilute sulfuric acid, which is separated from a carbon cathode in nitric or chromic acid by a porous pot. A single element delivers an electric potential of about 1.9 V.

tions at the same electrophoretic velocity toward their respective electrodes, a view shaped by the theories of Th. von Grotthuß, H. Davy, and M. Faraday. However, this view was challenged by the results of recent experiments, which had shown that the solutions of some strong electrolytes after electrolysis had different concentrations near the two electrodes, in the so-called electrode compartments. These findings were incompatible with the established theory. Wilhelm Hittorf drew his conclusions from these observations and argued that the earlier view was based on a fallacy. Instead, he derived a hypothesis according to which anions and cations actually move electrophoretically at different speeds. In his opinion, concentrations can only differ if one type of ion migrates faster than the other. He related the change in concentration in the electrode compartments to the current that an ion type transports relative to the total current which flows during electrolysis. He termed this fraction of the current, which is equal to the ratio of the velocity of a particular ion to the sum of the velocities of both ion species "Überführungszahl", i.e., transference or transport number of an ion in a given electrolyte. Regrettably, the transference number expressed only the velocity of an ion relative to its counterion, but did not give the magnitude of the absolute velocity.

At the end of the 1860s, Friedrich Kohlrausch began researching the conductivity of electrolytes, a topic that would subsequently occupy him throughout his life. He found that conductivity decreased with decreasing concentration of the electrolyte, but more relevant to him were the conductivities normalized to their concentrations. He observed that the ratio k/m of conductivity, k, and the equivalent or molar concentration, m, of the electrolyte increased with increasing dilution. He quoted k/m as the equivalent and molar conductivity, in modern notation, Λ,[76] the quantity which turned out to be central for his further research.

Kohlrausch found that two types of electrolytes can be distinguished. With strong electrolytes, such as neutral salts, the value k/m decreased only slightly with increasing concentration. With weak electrolytes such as acetic acid and ammonia, on the other hand, there was no such dependence, since k/m remained at low values at low dilutions but rose steeply within a certain narrow range of decreasing concentration.

He subsequently focused his research on strong electrolytes in very dilute solutions, preferably but not exclusively on those consisting of single-charged ions. He was able to show that ion and counterion of an electrolyte migrate independently of each other and that an ion, regardless of which electrolyte it comes from, always has the same "molecular" – now molar – ion conductivity. This was the law of independent ion migration, also quoted as the 1st Kohlrausch law, which he derived in 1879. In determining Λ at different concentrations, Kohlrausch formulated an empirical law which stated that Λ decreases linearly with $m^{1/2}$ for very dilute solutions. It is known as the 2nd Kohlrausch law and was called by him the "Quadratwurzel Gesetz (square root law). The extrapolation of the ion concentration to limiting conditions, i.e., to concentrations approaching zero, led to an ion-specific and concentration-independent variable, the limiting ion conductivity. It is little known that the 2nd Kohlrausch law was preceded by the relationship between Λ and the cubic root, $m^{1/3}$, which applied to a larger concentration range.

The ion velocities and their mobilities, that is, their drift speed at a unit field strength, could be calculated by Kohlrausch by combining Hittorf´s transfer numbers with conductivity data and the law of independent ion migration. However, their knowledge did not contribute to a deeper understanding of the form in which ions exist in solutions.

It also did not indicate how they drift in the electric field trough the solution, although a connection with the viscosity of the solution and with the frictional resistance has already been assumed. It must be remembered that the generally accepted concept before the late 1880s was not very different from the one introduced by von Grotthuß about eight decades ago. It was still based on the conjecture that an ion only exists in solution in an electrically neutral assembly with a counterion, which needs an electric field to divide. Rudolf Clausius, on the other hand, argued in 1857 that ions in solutions exist in free form as a result of their thermal energy even in the absence of an external electric force, although, as he assumed, only to a small extent.[77]

We do not wish to diminish the significant contributions of many other scientists to ion migration, but it would not be appropriate to quote names selectively. Certainly we must cite the early contributions of G. Fechner, but also refer to the later measurements of ion migration velocities by those mentioned in footnote [57] who mainly used the moving boundary method for their determinations.

[76] For the sake of better readability, we replace the written terms by symbols and restrict ourselves to strong 1:1 electrolytes with single-charged ions. The molar conductivity of the electrolyte solution is Λ, that of the ion species λ. The molar concentration is symbolized by m.

[77] This latter aspect of Clausius´ theory, the low fraction of free ions, was retrospectively disproved in 1889 by Wilhelm Ostwald and Walter Nernst in their paper entitled "*Über freie Ionen*" (*On free ions*) by the conflictive consequences to the law of thermodynamics and by subtle experiments.[179]

However, we consider Kohlrausch a key figure in the field, having done pioneering work for more than four decades, longer than others, although his greatest achievements in the field of electrophoretic properties were ultimately in the area of strong electrolytes. At the end of Kohlrausch's research, which lasted until about 1910, two questions remained unanswered for him. The first question was why the values of strong electrolytes increase with increasing dilution to a limit at concentrations close to zero. Kohlrausch hypothesized that under the limiting, but only under these conditions, possible ion-counterion interactions tend to cease completely. But the second open question, namely why compounds of the 2nd class, weak electrolytes like acetic acid, deviate so much from the usual behavior of the strong electrolytes, could by no means be answered conclusively with this assumption.

The answer to the first question was given at the begin of the *Short 20th Century*[78] by Lars Onsager,[190, 191] based on the theories of Peter Debye and Erich Hückel.[192, 193] But Kohlrausch could have seen the solution to the second problem as early as the 1890s, if he had not been so skeptical of the theory of electrolyte dissociation Svante Arrhenius published in 1887. In fact, this dissociation theory not only provided the plausible answer to this second question but moreover, represented the next, major step toward the modern physical chemistry of electrolyte solutions. This pioneering theory and its consequences in the last decades of the *Long Nineteenth Century*, or, as we term it, the "*1st period of electrophoresis*," which ended in 1914 with the first utilization of electrophoresis as a method for separating compounds in mixtures will be the subject of the following Part 4.

We attach great importance to close this chapter of scientific research not without pointing out that electrophoresis of ions in free solutions in capillaries was performed for the first time in its history in the second half of the *Long Nineteenth Century*, in four times between 1861 and 1895. The last time this happened was in 1895, when F. Kohlrausch and A. Heydweiller applied direct current instead of alternating current to liquids filled in glass capillaries and observed the movement of the boundaries of ion zones caused by unexpected resistance changes. One could speculate whether this phenomenon led him to derive his important "beharrliche Funktion" in 1897, the "regulating function". In 1889, W. Ostwald and W. Nernst investigated whether free ions are present in solutions according to Clausius' theory. They used the capillary of a Lippmann electrometer for their experiments. In 1865, W. Beetz investigated ion migration in capillaries because he doubted the results of Edmond Becquerel, who wanted to compare the conductivities of liquids in capillaries with those of metal wires. In any case, it is recognized that the priority is owned by Edmond Becquerel, since he first performed capillary electrophoresis of ions as early as 1861.

[78] The "*Short Twenties Century*," popularized by the noted British historian Eric Hobsbawm (1917 – 2012) in his book *The Age of Extremes*, covers the period between the beginning of World War I in 1914, i.e., at the end of the *Long Nineteenth Century*, until the beginning of the collapse of the USSR in 1989. For details, see Part 1 of our review series.

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Historical Article

The Early History of Polyaniline II: Elucidation of Structure and Redox States†

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Abstract. Polyaniline, one of the primary parent conducting polymers, is a quite old material with a history dating back to 1834. With the distinction of being the oldest known fully synthetic polymer and successfully commercialized as several popular cotton dyes in the 1860s, this material was originally known by the name of its black dye, aniline black. Of course, throughout this early history, the chemical identity and structure of these early polyaniline products were completely unknown, and it was not until the 1870s that initial attempts began to reveal various structural aspects. The current report will present a detailed historical account of the efforts to determine the structures of these early aniline oxidation products over the time period of ca. 1870-1915. In addition to the identity and structure of specific products, studies revealing the interconversion of one species to another via both redox and acid-based processes will also be discussed, with these collective efforts resulting in a comprehensive model of these materials that has remained essentially unchanged to this day.

Keywords: polyaniline, aniline black, emeraldine, nigraniline, structure elucidation.

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INTRODUCTION

Polyaniline is one of the most commonly studied parent conjugated polymers (Figure 1),[1-8] with its oxidized emeraldine form (Figure 2) representing one of the earliest examples of a conducting organic polymer. While such conducting polymers generated from the oxidation (*p*-doping) or reduction (n-doping) of conjugated polymers are generally viewed to be quite modern materials,[1,3-7] electrically conductive polymers actually date back to the early 1960s. Conjugated polymers as a whole are even older, with a history dating back to the early 19th century.[9-14]

Within this long historical record, polyaniline holds the distinction of being the earliest known conjugated polymer and dates back to 1834 with the work of the German chemist F. Ferdinand Runge (1794-1867), who treated protonated aniline salts with various oxidants to generate green and

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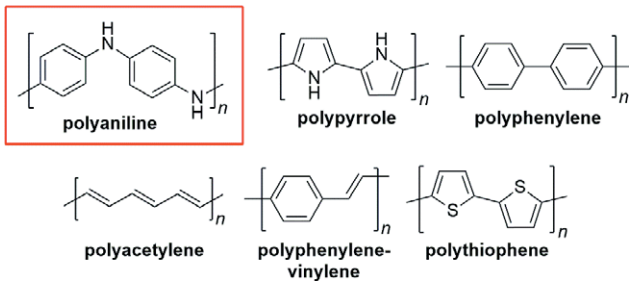


Figure 1. Common parent conjugated organic polymers.

black materials.[15] As such, it predates the 1839 report of Eduard Simon (1789-1856) detailing the generation of the material now identified as polystyrene,[16] making polyaniline also the oldest known fully synthetic organic polymer.[12,17] Of course, the modern name polyaniline was not introduced until the 1960s,[18-20] prior to which it was referred to by various color-based names, the most common of which was *aniline black*. This emphasis on color was largely due to the fact that the primary application of early polyaniline was as green, blue, and black dyes for cotton, with the commercialization of these dyes dating back to 1860.[10-13] At the same time, it is important to note that the chemical identity and structure of these early polyaniline products were unknown at the time and it was not until the beginning of the 20th century that deeper knowledge of the material's composition began to take shape.

The structural details of polyaniline are complicated by the fact that it is the only member of the common conjugated polymers to exhibit both protonated and free-base forms (Figure 2). In addition, while conjugated polymers are all redox active materials, thus leading to conducting polymers in their non-neutral redox states, polyaniline exhibits multiple known oxidative

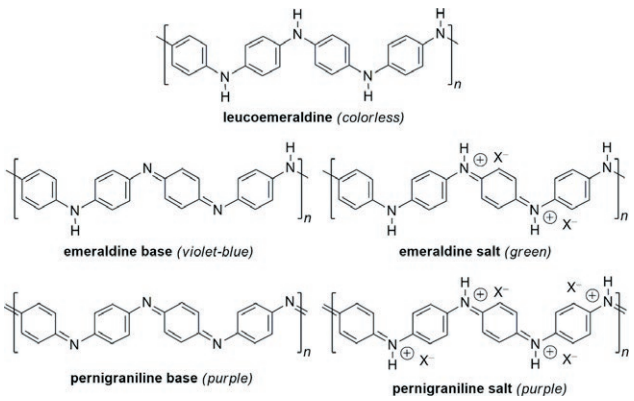


Figure 2. Various common forms of polyaniline.

states, each with distinct properties and characteristics. Although previous reports have presented an in-depth early history of polyaniline up to ca. 1870,[13,14] the current report will present a detailed historical account of the efforts to determine the structures of these early aniline oxidation products, beginning with attempts in the 1870s to reveal the mechanistic processes involved in their synthesis. In addition to the identity and structure of specific products, studies revealing the interconversion of one species to another via both redox and acid-based processes will be discussed, with these collective efforts of ca. 1870-1915 resulting in a comprehensive model of these materials that has remained essentially unchanged to this day. The beginnings of these efforts can be traced to a particularly noteworthy report by Heinrich Rheineck in 1872.[21]

RHEINECK AND NIGRANILINE

Although Heinrich Rheineck played an important role in the history of polyaniline, very little is actually known about him. Originally from Neckarsulm, Germany (near Stuttgart), he enrolled in the study of pharmacy at the University of Tübingen in the fall of 1860.[22] At Tübingen, he carried out research under Adolph Strecker (1822-1871) on the action of sodium on allantoin, resulting in a single paper published in 1865.[23] He then worked in Hohenheim as an assistant chemist in agricultural chemistry for several years,[24] but had moved to Hagen, Germany in North Rhine-Westphalia (near Dusseldorf) by 1871.[24-26] Here he studied aspects of inorganic dyes such as Prussian blue.[26] His time in Hagen was short, however, and by 1872, he had moved to nearby Elberfeld (ca. 30 km to the southwest),[21,27] most likely employed by one of the smaller aniline dye companies located there. It was at Elberfeld that Rheineck then published his seminal work on aniline black.[21] This paper attracted a fair amount of interest and it was republished a number of times.[27-29]

Rheineck began the discussion of his study by stating that like other aniline dyes, aniline black is produced by the oxidation of aniline, thus resulting in the molecular condensation of repeated aniline units. Furthermore, these oxidation products are still of a basic nature, with aniline black a definite base. He then proposed that this base be called *nigraniline* (Latin *nigrum* "black" + *aniline*) in analogy to the name *rosaniline* for aniline red.[21]

In order to support these conclusions, he produced samples of aniline black following common conditions for its production on fabrics (aniline hydrochloride, potassium chlorate, and copper chloride). This aqueous

mixture was allowed to evaporate in a porcelain dish and repeatedly moistened until a dry, velvety black powder was produced, after which the product was washed with hot water. As it was well known that this material appeared dark green on fabric and turned dark blue-violet after treatment with alkalis, Rheineck proposed that the blue-violet material was a free base, with the initial green material the corresponding hydrochloride salt. To confirm this, he treated the initially produced aniline black with either soda or ammonia to remove hydrochloric acid and generate what he viewed to be the free base.[21] This base could then be successfully used to remove acid from aniline salts. That is, if the isolated free base was produced on a piece of cotton, and then treated with aniline salts in the absence of oxidant, the fabric immediately turned green. Treating this again with alkali then returned the fabric to the original blue-violet color. In addition, he showed that the green hydrochloride salt could also be treated with sulfuric acid to release hydrochloride fumes and give a violet solution. When diluted, this solution again gives a black-green precipitate, which he was confident was the sulfuric acid salt.[21]

Rheineck felt that these results confirmed his view that the blue-violet material was a free base (nigraniline), which generated green salts when treated with acid. However, he decided not to follow through with more detailed studies, stating:[21]

> For lack of opportunity and facilities, I have to refrain from further elaboration on this interesting subject in scientific terms, and make other chemists aware of it, to which a well-established laboratory is available.

As such, this remained his only publication on aniline black and he left it to others to expand upon his initial efforts. One such to carry out further efforts was the German chemist Rudolf Nietzki (1847-1917), who reported various studies on aniline black starting in 1876.[30,31]

NIETZKI AND OXIDATIVE DEGRADATION

Rudolf Hugo Nietzki was born on March 9, 1847, in Heilsberg, Prussia (now Lidzbark Warmiński, Poland) to a Protestant family.[32-35] His father, Karl Johann Emil Nietzki, was a pastor, rector and writer.[32,33] In 1854, the family moved to the Prussian town of Zinten (now Kornevo, Russia), where his father served as pastor to the small town.[35] Initially educated by his father, Nietzki was sent to complete his studies at the gymnasium in Königsberg (now Kaliningrad, Russia), as Kinten was too small to have schools for higher education.[32,33,35]

Unhappy with his gymnasium education, he left before completion[32,35] and began the study of pharmacy, beginning with an apprenticeship in Zinten, followed by an internship in Kreuzburg, Prussia (now Kluczbork, Poland).[33,35] After passing the assistant examination in 1865,[32,33] he then worked in a pharmacy in nearby Hirschberg (now Jelenia Góra, Poland),[33,35] before additional studies at the University of Berlin in 1867.[32,33,35] In 1870, he passed the state pharmacy examination[35] and soon after was called to serve as a military pharmacist in the Franco-Prussian War,[32,33,35] during which he was captured by the French and held as a prisoner of war.[33,35] Following the end of the war in 1871, he was offered a position as private assistant to August Wilhelm Hofmann (1818-1892) at the University of Berlin.[32,33,35] During his spare time, he also carried out some research in plant chemistry,[35] such that he was able to obtain his Dr. phil. at the University of Göttingen in 1874.[32-35] As he did not have a certificate of maturity from a gymnasium, he could not take the degree in Berlin.[35]

After obtaining his doctorate, he worked as a chemist for Matthes & Weber in Duisberg,[32,33,35] but then moved to the University of Leiden in 1876 to became first assistant in the laboratory there,[35] working under Antoine Paul Nicolas Franchimont (1844–1919)[32,33,35] and Jacob Maarten van Bemmelen (1830-1911).[35] In 1879, he began working as a research chemist in the laboratory of Kalle & Co. in Biebrich, Rhineland-Palatinate,[32,33,35] but left in 1883 to rent a place in the laboratory of Dr. Schmidt, in Wiesbaden, with the desire to carry out independent industrial and scientific studies.[35] In the spring of 1884, he moved to Basel, Switzerland, to introduce processes he had developed to the dyestuffs company J. R. Geigy[33,35] and at the same time obtained space in the laboratory at the University of Basel.

At the University, Jules Piccard (1840-1933) quickly recognized the value of having a specialist in dye chemistry at Basel and offered to establish Nietzki as a privatdozent.[35] Nietzki completed his habilitation at the University of Basel on June 30, 1884, after which he was appointed professor extraordinarius in 1887.[32,33,35] He was made professor of chemistry in 1895[32-35] and as the university laboratory did not have enough spaces to meet the demand of students, Nietzki established an organic chemistry laboratory at his own expense, in a private house located in the rue du Rhin. The lab initially accommodated 20 students, but was later expanded to allow 36 students. During his third decade at Basel, rapidly developing arteriosclerosis hampered his ability to work and ultimately made laboratory manipulations very difficult.[35] As a result, he resigned as professor in March of 1911,[32,33,35] retiring to Freiburg im Breisgau, in

southern Germany, the following year.[35] After a long illness, he died at the Neckargemund Sanatorium on September 28, 1917.[32-35]

It was while at Leiden that Nietzki began reports of his investigations into the nature of aniline black in 1876, beginning with efforts to produce high purity samples for study.[30] Once satisfied with the quality of the samples of aniline black, he then began efforts to analyze its chemical composition. Such efforts resulted in the empirical formula $C_{18}H_{15}N_3 \cdot HCl$. It should be noted that this formula is very similar to that previously reported by Carl Julius Fritzsche (1808-1871) in 1843, although with lower Cl content.[13,14,36] As Nietzki had extensively purified his aniline black samples, it is quite possible that he had partially reduced his sample, thus inadvertently reducing the cationic content and thus the amount of Cl^- counterions. Two additional analysis papers then followed in 1876[31] and 1878[37], but these efforts focused primarily on the blue material generated by boiling isolated aniline black in excess aniline, which Nietzki viewed to be a form of phenylated aniline black. In the 1878 paper, he does conclude that the amount of Cl found during analysis is dependent on the methods of purification and drying of the sample.[37]

It was during the period between the second and third of these papers on the analysis, however, that Nietzki reported a discovery that would have far more impact on the eventual elucidation of the structural nature of aniline black. Thus, in an 1877 report,[38] he found that if aniline black is heated with potassium dichromate ($K_2Cr_2O_7$) in sulfuric acid, copious amounts of quinone was produced. To study the nature of this reaction, he suspended aniline black in dilute sulfuric acid and gradually added potassium dichromate. In the process, the black material was consumed, giving rise to a strong odor of quinone. The brown, liquid mixture was then separated by steam distillation to give a considerable amount of quinone, although not enough to correspond to the initial amount of aniline black. The residual content of the still was then evaporated to give a colorless crystalline material identified as hydroquinone. Thus, Nietzki concluded that aniline black was initially converted to hydroquinone, which was then oxidized to quinone (Figure 3). As aniline black is itself produced via the oxidation of aniline, Nietzki proposed that hydroquinone or quinone could be produced in this way from aniline, in which aniline black was then just an intermediate oxidation product. Treatment of aniline with dichromate in dilute sulfuric acid confirmed this view to be correct, with the ratio of hydroquinone to quinone dictated by the amount of oxidant applied.

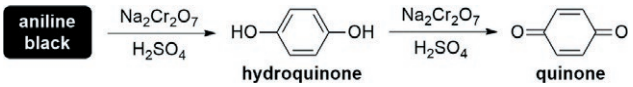


Figure 3. Nietzki's proposed oxidative degradation of aniline black.

Nietzki then followed this initial report with a second paper in 1878, here focusing on optimizing the isolated yield of quinone.[39] While he had previously found that hydroquinone could be produced in good yield, efforts to generate the final quinone always occurred in significantly lower yields, with the exception of attempts carried out at very small scale. As this led him to suspect that the quinone was undergoing further reactivity under heat and oxidation, he steam distilled samples of pure quinone in the presence of oxidant, which resulted in the generation of hydroquinone and a resinous product. He then repeated the process without the oxidant, again getting similar results, which led to the belief that quinone was undergoing condensation under heat. Thus, rather than the application of steam distillation, he found that near quantitative amounts could be generated by the slow addition of potassium dichromate to cooled sulfuric acid solutions of aniline, after which the quinone was isolated by ether extraction.

Of course, the generation of quinone and hydroquinone via the oxidative degradation of aniline black provided some clues as to structural aspects of the initial aniline material. However, this did not seem to be a significant focus for Nietzki, who seemed much more interested in this process as an easy and effective method for the generation of quinone. The next significant step towards an understanding of the structures involved then came from one of the original developers of commercial black aniline dyes, Heinrich Caro (1834-1910).

CARO AND CRITICAL INTERMEDIATES

Heinrich Caro (Figure 4) was born in Posen, Prussia (now Poznań, Poland) on February 13, 1834.[13,40] The family moved to Berlin in 1842, at which point Caro entered the Köllnische Realgymnasium there.[40] He continued his studies at the Köllnische Realgymnasium until 1852, after which he attended the Königliches Gewerbeinstitut (Royal Technical Institute), which trained students for industry.[13,40-42] At the same time, he was also attending lectures at Berlin's Friedrich-Wilhelms-Universität (now the Humboldt University of Berlin).[13,40-42]

Towards the end of his studies, Caro was encouraged to focus on subjects related to printing and dyeing,

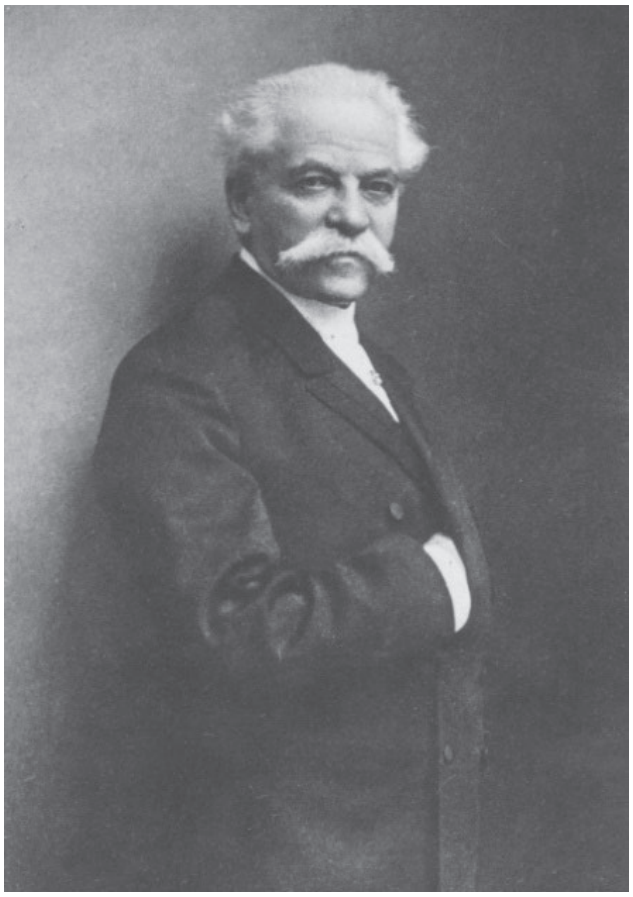


Figure 4. Heinrich Caro (1834-1910) (Edgar Fahs Smith Memorial Collection. Kislak Center for Special Collections, Rare Books and Manuscripts. University of Pennsylvania).

as discussion had begun about setting up a state school for the training of technicians in this field, which would thus require teachers.[40] Thus, in April of 1855, Caro took a modest appointment with C. &. F. Troost, a calico printing company in Mülheim an der Ruhr, where he mainly performed analytical work.[13,40,42] In March of 1857, Caro was then sent on a study trip to England, where he was instructed to visit a large number of printing and dyeing factories.[13,40-42] In particular, he visited Roberts, Dale & Co, having previously met the owner John Dale in 1854 during a trip to Germany.[40,41] After Caro's return to Germany, the decision was made to build a larger factory and to transform C. &. F. Troost into a corporation, becoming Luisenthaler Aktiengesellschaft of Mülheim.[40] During this same period, Caro carried out his military service in 1857-1858.[40]

The following year, Caro decided to try his luck in England, moving there in November of 1859. Although his initial efforts were unsuccessful, he was ultimately able to obtain a position with John Dale at the Cornbrook Chemical Works of Roberts, Dale & Co. in Manchester.[40-43] It was during this time that Caro developed a process for making aniline purple in 1860, from which a black residue was isolated as a byproduct. This residue provided an excellent fast black dye for printing on cotton, with this aniline black then commercialized by Roberts, Dale & Co. in 1862.[13,41,42] At least partially due to declining health, Caro dissolved his partnership with Roberts, Dale & Co. in October of 1866, after which he returned to Germany.[41,42]

Once back in Germany, Caro took a post in the laboratory of Robert Bunsen (1811-1899) at the University of Heidelberg, and began private consultancy work, primarily for the newly formed Badische Anilin und Soda Fabrik (BASF).[41] His work with BASF grew to the point that he was hired as a technical director by the end of 1868.[40-43] At BASF, he oversaw the development of various new dyes, including artificial alizarin, eosin, methylene blue, and azo dyes, as well as the initial stages of the indigo synthesis.[41,42] His various contributions to the dye industry resulted in Caro being awarded an honorary doctorate by the University of Munich in 1877.[42] It was also during this time period that Caro started to become involved in the development of German patent law, which had only been recently introduced.[42,43] Caro was then appointed to the company's board of directors in 1884.[43] By the end of 1889, however, the strain of his activities in the fields of both chemistry and patent law, as well as his duties as director, lead to an end of his direct involvement in the work at BASF,[42] although he remained active on the company's supervisory board.[42,43] After a short illness, Caro died on September 11, 1910 in Dresden.[13,40,43]

It was towards the end of his career, in 1896, that Caro published studies that followed up on a previous statement by Nietzki concerning the production of a yellow species from the oxidation of aniline in cold, aqueous alkaline solutions.[44,45] Initial efforts showed that the addition of a permanganate solution to alkaline, aqueous aniline solution resulted in the production of a green solution coupled with precipitation.[44] Filtration isolated a solid mixture of MnO_2 and azobenzene (Figure 5), leaving a yellow solution. Optimized methods utilized a 2% solution of potassium permanganate to an NaOH solution of aniline with vigorous stirring.[45]

The yellow filtrate was then treated with iron sulfite to give a colorless solution, which was then filtered, and evaporated to remove unreacted aniline.[44,45] On cooling, a black tar separated, which was then boiled with dilute sulfuric acid to result in the crystallization of a colorless sulfate salt which was insoluble in cold water. The cor-

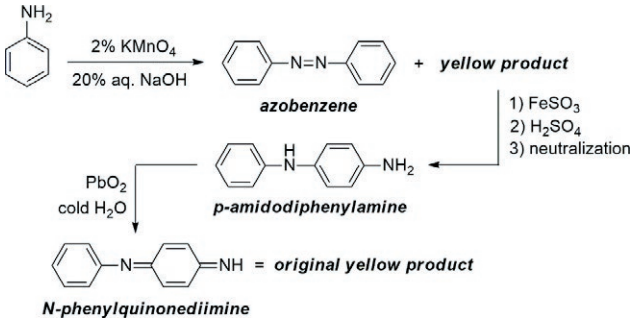


Figure 5. Caro's synthesis and identification of the intermediate oxidation product *N*-phenylquinonediimine.

responding base was then crystallized from ligroin as colorless, flat needles which were found to be the known species *p*-amidodiphenylamine (Figure 5).[44,45] Oxidation of this base with PbO_2 in cold water then resulted in the isolation of yellow crystals, which were ultimately determined to be *N*-phenylquinonediimine.[45] This final product was believed to be the identity of the original yellow species. Caro recognized that *N*-phenyl-quinonediimine and azobenzene were isomeric species, with both isomers formed during the alkaline oxidation of aniline. The exact mechanism of their formation, however, still remained unknown.[45] Further efforts were then continued in 1906 by the German chemist Richard Willstätter (1872-1942).[46]

Figure 6. Richard Willstätter (1872-1942) (Edgar Fahs Smith Memorial Collection. Kislak Center for Special Collections, Rare Books and Manuscripts. University of Pennsylvania).

WILLSTÄTTER AND LINEAR OLIGOANILINE STRUCTURES

Richard Willstätter (Figure 6) was born on August 13, 1872, in Karlsruhe, Germany to Jewish parents.[47-49] After two years in the Gymnasium at Karlsruhe, the family moved to Nürnberg in 1883, where he entered the Realgymnasium with the goal of a commercial career.[48] He then moved to Munich in October 1890, where he studied at the University of Munich while also attending lectures at the Technische Hochschule.[48,49] Although Adolf Baeyer (1835–1917) was Chair at Munich, the bulk of Willstatter's studies were under Eduard Buchner (1860–1917), Johan Rupe (1866-1951) and Eugen Bamberger (1857–1932). He took the pre-doctorate examination in 1893 and was assigned to Alfred Einhorn (1856-1917) as a research student.[48,49]

Willstätter started his independent work in 1894, after which he became privatdozent in 1896.[48-50] He was then made professor extraordinarius and Head of the Organic Section in 1902.[48,50] In 1905, he accepted the Chair of Chemistry at the Eidgenössische Polytechnische Schule in Zurich (now ETH Zurich).[47-51] He was then appointed Director of the new Kaiser-Wilhelm-Institut for Chemistry in Dahlem (now the Max Planck Institute for Chemistry), moving to Berlin in 1912.[47-51] He then returned to Munich in 1915 to succeed Baeyer, becoming professor and Director of the State Chemical Laboratory. That same year, he was also awarded the Nobel Prize for Chemistry for his work on chlorophyll and plant pigments.[47-51] Unfortunately, rising antisemitic views in Germany ultimately led to his resignation on June 24, 1924,[48,49] after which he retained a room to continue some limited research.[48,50] A spate of attractive offers from other institutions followed, but all were declined.[49,51] Recognition for his contributions also continued, including the Davy Medal of the Royal Society of London in 1932 and the Willard Gibbs Medal of the Chicago Section of the American Chemical Society in 1933.[48,50] In late 1938, the Gestapo searched his house and he was later ordered to leave the country, resulting

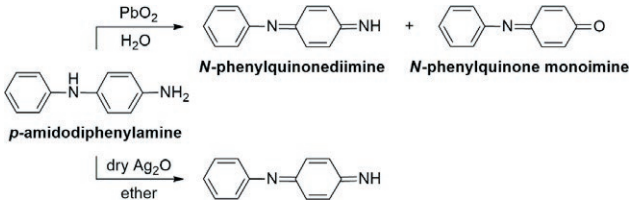


Figure 7. Oxidation of *p*-amidodiphenylamine.

in his move to Muralto-Locarno in southern Switzerland in 1939.[48-51] There, he died of a heart attack on August 3, 1942.[49-51]

Willstätter began his investigation of aniline black and aniline oxidation by revisiting Caro's oxidation of *p*-amidodiphenylamine by lead oxide as discussed above (Figure 5). In analyzing the isolated oxidation product, he found the nitrogen content to be lower than expected for the *N*-phenylquinonediimine proposed by Caro, ultimately concluding that carrying out the reaction in water resulted in a portion of the oxidation product to be hydrolyzed to the corresponding *N*-phenylquinone monoimine (Figure 7), which co-crystallizes with the diimide.[46] In contrast, he found that if the oxidation was carried out in an ether solution using dry silver oxide, pure *N*-phenylquinonediimine could be obtained.

Willstätter then continued with polymerization studies of *N*-phenylquinonediimine via treatment with acid.[46] The yellow material first turned red-brown upon protonation, after which it eventually turned into a dark green product. This green product was referred to as *emeraldine* in reference to the commercial name of the green aniline-based dye developed by Frederick Crace-Calvert (1819-1873) and coworkers in 1860.[13] Based on a suggestion from Nietzki, it was found that the same material could be produced more easily by oxidizing *p*-amidodiphenylamine with iron chloride in acidic media. Similar results were also achieved using hydrogen peroxide and a catalytic amount of $FeSO_4$.[46]

From these products, a blue species was isolated that was viewed to be the emeraldine base, also known as the blue aniline-based dye *azurine*. Analysis of the crystallized compound led to a formula of $C_{24}H_{20}N_4$, which was concluded to have been formed from the polymerization of two molecules of *N*-phenylquinonediimine.[46,52] Benzene solutions of the blue base were then oxidized with PbO_2 to give a red product with two less hydrogens ($C_{24}H_{18}N_4$). Both the blue and red products could be reduced to give the colorless leuco base ($C_{24}H_{22}N_4$), which could not be reduced any further.[46] As in the case of the oxidation of *p*-amidodiphenylamine in water (Figure 7), oxygen-terminated byproducts were also found in the blue, red, and leuco base species here.

Efforts were then made to determine the structures of the blue, red, and leuco base species, beginning with consideration of what type of bond might link the two *N*-phenylquinonediimine units. Of the various new linkages considered, the only option that was viewed to be consistent with the observed results was the formation of a new C-N bond between a terminal nitrogen of one unit and a phenyl unit of the other. With the nature of the linkage limited to a simple C-N bond, there still remained various possible structural motifs as illustrated in Figure 8.[46] Of the three possible structures given, it was viewed that the two branched structures (II and III) should be easily converted into azine species (IIb and IIIb) and thus efforts to find evidence of azine content in the blue species was pursued. As no indication of azine content could be found, it was determined that the linear structure (I) was the most reasonable structure for the blue species,[46,52] which was also consistent with the initial formation of *p*-amidodiphenylamine from aniline. From this determination, the structures of the three species and their transformations via oxidation or reduction can be summarized as shown in Figure 9.[46]

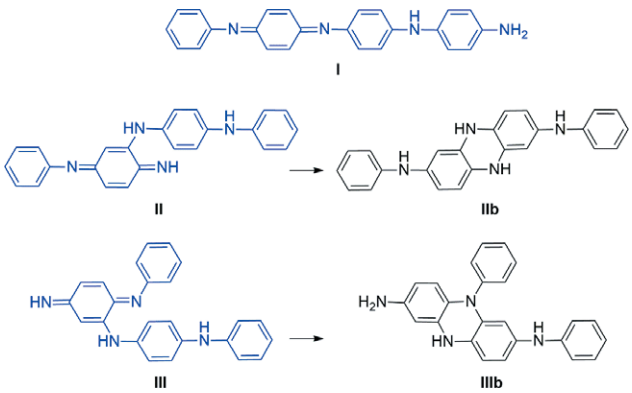


Figure 8. Possible structural motifs for the isolated blue product.

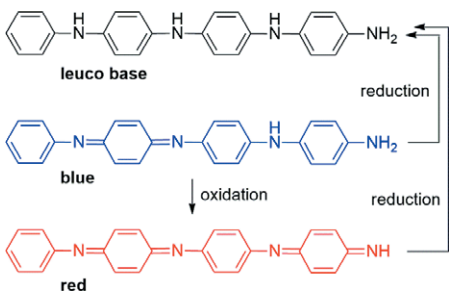


Figure 9. Structures and interconversions of isolated species.

Willstätter viewed his various emeraldine products as intermediates in the formation of aniline black. However, it was found that of the three species given in Figure 9, only the isolated red species could be converted directly to an insoluble black material.[46] In this way, it was found that the red product underwent further polymerization upon heating or treatment with dilute acids to give a black product referred to by Willstätter as *polymerization black*. He acknowledged that the properties of the aniline black prepared from the red intermediate did depend on the exact conditions applied, but examples that produced aniline black without byproducts gave a composition of $(C_6H_{4.5}N)_x$. Thus, he concluded that this composition best represented that of aniline black. As his aniline black was produced from the red intermediate consisting of four units (i.e. $x = 4$), he concluded that the smallest possible structure for aniline black was where x equaled a minimum of 8, with the simplest possible formula being $C_{48}H_{36}N_8$.[46]

Willstätter recognized that this was not a definitive determination of the structure of aniline black, admitting in a second paper published in 1909:[52] "The way in which the aniline residues are linked in [aniline] black has not yet been determined." In fact, the German chemist Hans Theodor Bucherer (1869-1949) had published a paper in 1907 that refuted the proposed structures in Willstätter's initial report, stating that only azine-type structures could explain the significant stability of the aniline black.[53] To further support the linear structures given in Figure 9, Willstätter subjected samples of aniline black to strong oxidation, which had previously been shown by Nietzki to decompose the black material to quinone.[44,45] Various methods were thus studied in order to maximize the experimental yield of quinone from aniline black.[52] The resulting experimental yields were then compared to the theoretical yields of quinone possible from the various proposed structures for aniline black at the time, which showed that the experimental values were ca. 95% of the theoretical value expected for the linear structure proposed by Willstätter, but much higher than that expected for the alternate branched or azine-based structures. In the same study, a more detailed elemental analysis was also performed, resulting in a more precise average composition of $C_{5.97}H_{4.55}N$. Based on this composition, it was thus proposed that aniline black had a composition consisting of units of the three-fold quinoid derivative given in Figure 10.

In a second 1909 paper,[54] Willstätter continued the study of aniline black to determine the presence and interconversion of two different quinoid species, both

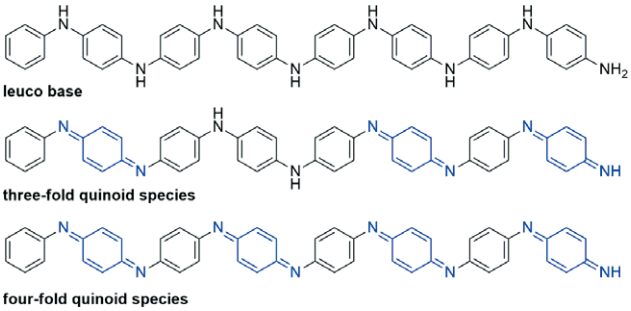


Figure 10. Proposed oxidative states representative of aniline black (quinoid units highlighted in blue).

of which were derived from the same leuco base (Figure 10). In addition to the three-fold quinoid derivative previously proposed, he now added the fully oxidized four-fold quinoid unit, which could be produced from the three-fold species via further oxidation with hydrogen peroxide. This fully oxidized species was described as blue-black, with salts giving a dark green color. The content of these two oxidized species in aniline black was viewed to be dependent on the oxidation conditions applied. The three-fold quinoid species, which gave a blue-colored base and green materials as the protonated salts, was viewed to represent the traditional material emeraldine. In contrast, the polymerization black previous generated from the heating of his red intermediate (Figure 9) was viewed to be closer to the four-fold quinoid species.

This was then followed up with a 1910 paper in which the gradual reduction of aniline black was studied via treatment with phenylhydrazine carbamate.[55] In this way, the initial aniline black (viewed to be primarily the three-fold quinoid species) was found to progress through three phases. The initial reduction was observed at 30-45 °C, resulting in a transition from a dark blue base to light blue color. Further heating resulted in another transition at ca. 80 °C to give a gray material. Finally, a colorless product was obtained at temperatures above 120 °C. The leuco base produced in this manner converted back to the black material under atmospheric oxygen in the presence of a small amount of ferrous salt.

Concurrent with the publication of Willstätter's study on the reduction of aniline black, the English industrial chemists Arthur G. Green (1864-1941) and Arthur E. Woodhead began reporting competing studies. While the first of these papers was reported in 1910,[56] two solo works by Green had also been reported the previous year.[57,58] At least initially, Green had very different ideas concerning the structure of aniline blacks.

GREEN, WOODHEAD, AND FURTHER REFINEMENT

Arthur George Green (Figure 11) was born in 1864, in the town of Ealing, located in West London, and was educated at Lancing College, Sussex. After matriculating in 1880, he entered University College, London.[59,60] In his first year there, he won the gold medal in the junior practical chemistry class and the Clothworkers' Exhibition in chemistry; the next year he won the gold medal for chemical analysis, and in 1883 the Tuffnel Scholarship.[59,61] At the College, Green carried out research under lecturers Henry Forster Morley (1855-1943) and Richard John Friswell (1849-1908),[60,61] and worked as a volunteer during college vacations in the laboratory of Messrs. Williams Bros., aniline dye manufacturers of Hounslow.[60]

Green commenced his industrial career in June 1885, as research chemist with Messrs. Brooke, Simpson & Spiller, Ltd.[59-61] at the Atlas Aniline Dye Works in the east London neighborhood of Hackney Wick, where he had previously carried out his research with Friswell.[62] His initial career was successful and he was awarded the silver medal of the Royal Society of Arts in 1891.[59] He then left in 1894 to became manager at the Clayton Aniline Company, in Manchester.[59,60] In 1901, he decided to become independent and setup in London as a consultant, but accepted an invitation in 1903 to the Chair of Chemistry and Dyeing at the University of Leeds, a position made vacant by the death of John James Hummel (1850–1902).[59] Green was later elected to the Royal Society in 1915.

His time at Leeds was ultimately finite and he resigned the chair in March 1916 to became Director of Research at Levinstein Ltd. in Manchester.[59] He also gave part of his time during 1916-1918 to the College of Technology, Manchester, where he established the Dyestuffs Research Laboratory, with the assistance of Frederick Maurice Rowe (1891-1947), one of his past students.[59,60,63] During this period, Green continued to receive various accolades. He was elected to the Livery of the Worshipful Company of Dyers in 1918 and he received the Dyers' Company gold medal three times, in 1909, 1914 (with W. Johnson), and 1923. He was the Society's Perkin medalist in 1917 and received an honorary M.Sc. from the University of Leeds.[59]

In 1919, Levinstein, Ltd. merged with British Dyes to become the British Dyestuffs Corporation, Ltd, after which Green resigned his position as Director of Research in 1923. At this time, Green returned to his private practice which was considerable both in Europe and America.[59] In 1936, he returned to became consultant to the Dyestuffs Group of Imperial Chemical Industries Ltd., which was established in December 1926 from the merger of British Dyestuffs Corporation with three other British companies: Brunner Mond, Nobel Explosives, and the United Alkali Company. Five years later, Green died peacefully in his sleep at his home at Walton-on-Thames on September 12, 1941 at the age of 78.[59,60]

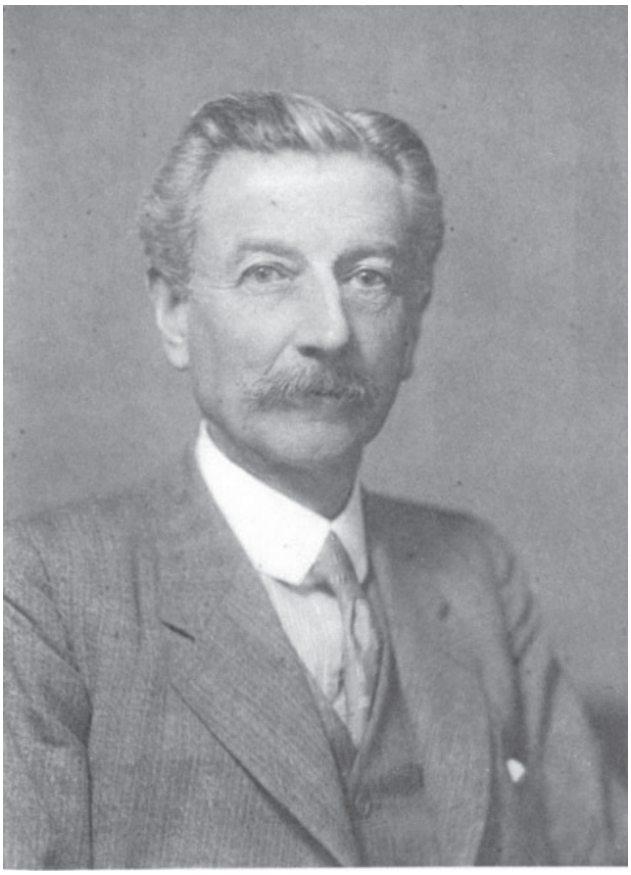
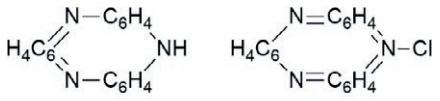


Figure 11. Arthur George Green (1864-1941) (*Obit. Notices Fellows R. Soc.* 1943, 4, 251–270; courtesy of JSTOR).

Green's earliest report on the structure of aniline black consisted of a short paper that presented proposed structures for both the emeraldine and nigraniline materials,[57] along with brief comments on the early report by Willstätter and Moore.[46] This was then followed up with a second report covering much of the same material in a bit more detail.[58] As shown in Figure 12, both of Green's structures were cyclic species consisting of three aniline repeat units. It is also important to note that he never designates what the substitution geometry is on various bridging phenyl rings (i.e. ortho, meta, or para). In terms of the report of Willstätter and Moore, Green agrees with the empirical formula of $(C_6H_{4.5}N)_x$. but does not accept any of the rest. As stated by Green:[58]



emeraldine nigraniline

Figure 12. Green's initially proposed structures of emeraldine and nigraniline.

> The view expressed by these authors that the product of this so-called "polymerisation" must have a molecule in which x = at least 8, and is to be represented as a complex indamine with a long open chain, appears to me to be very difficult to reconcile with its properties (stability to acids, etc.).

By the publication of the first paper coauthored with Arthur Edmund Woodhead in 1910,[56] however, this stance seems to be somewhat moderated, stating that the work of Willstätter and his coworkers has added much to the knowledge of the complex oxidation products of aniline. Still, they point out that the results of Willstätter can really only apply to the primary oxidation products of aniline (emeraldine or nigraniline), while the most stable form of aniline black, commonly known as *ungreenable aniline-black*, must be an azine. Based on this view, it was then proposed:[56]

> that the term "aniline-black" should be restricted to the higher condensation products (ungreenable black), whilst the original names "emeraldine" and "nigraniline" should be retained for the primary oxidation products.

Green and Woodhead then went on to reinterpret some of Willstätter's previous results and attempted to provide additional data to present a more detailed model of the primary oxidation products of aniline. In the process, they retained the general linear octameric structures of Willstätter, even though they felt the question of linear chains vs. ring structures was still undecided. On this point, they state:[56]

> Assuming the correctness of the eight-nucleal structure for the primary oxidation products, it still remains an undecided question whether the aniline residues are to be regarded as united in an open or in a closed chain, but without attempting to decide this point we shall make use of the open-chain formulae to express provisionally the analytical results.

These efforts started with the preparation of emeraldine via multiple methods, converted to the corresponding base, and carefully purified to produce an initial material for study. This material was then dissolved in aqueous solutions of either acetic (80%) or formic acid (60%) to give yellowish-green solutions. The addition of a very dilute solution of chromic acid then resulted in the color of the solution changing first from green to pure blue, and then to violet with the further addition of oxidant, which ultimately gave a violet precipitate.[56] If a very weak solution of sodium hydrogen sulfite was added to the violet solution, these color changes would occur in the opposite direction, from violet to blue to green. Stronger reducing agents (phenylhydrazine, sodium hyposulfite, or titanium trichloride) would convert the green solution to the colorless leuco base. As the initial solution began with the emeraldine, the next sequential colored solution via oxidation was designated nigraniline, with the name *pernigraniline* (Latin *per* "through, entirely, utterly" + *nigraniline*) proposed for the final violet solution. It should be noted here that the prefix per- is commonly used in chemistry to denote the highest known oxidation state of various species (i.e. persulfate or permanganate) and thus is added to nigraniline here to denote the fully oxidized form. In a similar fashion, the parent leuco base was given the name *leucoemeraldine* (i.e. the leuco base of the original emeraldine).

Their analysis then continued with efforts to determine the quantity of hydrogen required for conversion of emeraldine into leucoemeraldine via titration of the initial acetic acid solution with titanium trichloride.[56] From these measurements, it was determined that the emeraldine corresponded to a diquinoid species. In a similar manner, the quantity of oxygen consumed in the conversion of emeraldine into nigraniline was then studied by titration of the initial acetic acid solution with a standard solution of chromic acid. In this way, it was concluded that the conversion of emeraldine to nigraniline was the introduction of one additional quinonoid group. This then led to the series as outlined in Figure 13.[56] As the difference between emeraldine and leucoemeraldine was two quinoid units, the additional proposed single quinoid containing species was added between these in the series, which Green gave the name *protoemeraldine* (Greek *prōtos* "first" + *emeraldine*) to denote the first oxidized form.

WILLSTATTER'S RESPONSE AND GREEN'S REBUTTAL

Willstätter then responded to the work of Green and Woodhead in an additional 1911 paper.[64] While he felt that the proposed renaming of the structures provided no improvement over his previous descriptive nomenclature, it was the contradicting views on the quinoid content of emeraldine that dominated his response. The pri-

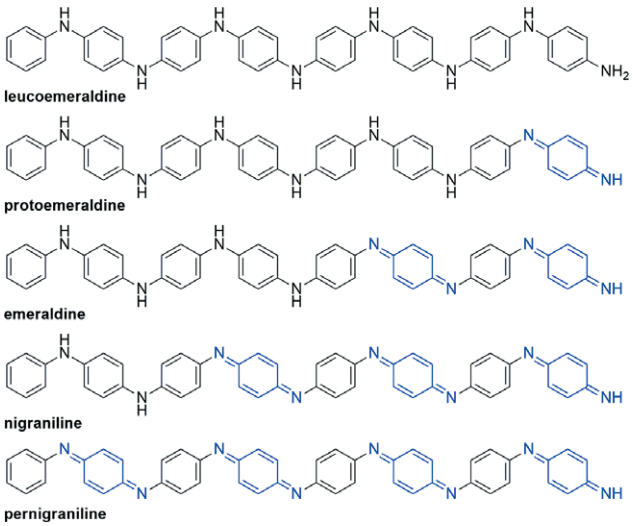


Figure 13. The full series of aniline oxidation products as proposed by Green and Woodhead in 1910.

mary point raised by Willstätter was that from their previous attempts at reducing materials to the leuco base, the titanium trichloride required higher temperatures for full reduction than that applied by Green and Woodhead. As such, he felt that the leucoemeraldine reported by Green was actually the monoquinoid product, which he felt was also supported by their description of a "pale brown amorphous powder", when it should be completely colorless. Such an assignment would then remove any differences between the studies of the two groups.

Green and Woodhead then provided their own response to Willstätter in 1912.[65,66] Here, they refute Willstätter's claim that full reduction to the leucoemeraldine cannot be accomplished with titanium trichloride at lower temperatures and highlight various points in Willstätter's work that they view as problematic. "In order to place the matter beyond doubt", attempts were made to further reduce their leucoemeraldine samples with titanium trichloride at high temperature. Here, leucoemeraldine was prepared from the reduction of emeraldine by phenylhydrazine at a low temperature, after which the leucoemeraldine was treated with boiling titanium trichloride. As the results were not consistent with the further reduction of a mono quinoid species to the full leuco base, it was concluded that their original conclusions were fully supported.

It does not seem that Willstätter had anything further to say on the subject, at least not in the chemical literature. As such, the debate was generally considered decided. This is further supported by the fact that no further modifications to these structural models have been reported since.

CONCLUSIONS

Other than the modern understanding of the macromolecular nature of polyaniline, the final oxidative series as presented by Green and Woodhead still remain the currently accepted structural forms. Although the modern literature typically gives Green and Woodhead the credit for determining the structure and oxidative forms of polyaniline, with little to no mention of Willstätter, it is clear from the historical record that the primary structure determination was really accomplished by Willstätter. This is not to ignore the contributions of Green and Woodhead, who clearly made important corrections and additions to the oxidative series, as well as establishing the traditionally accepted nomenclature, but this was all accomplished by refinement of the previous structural models of Willstätter. Even at the end, Green was not convinced of the linear nature of these materials and only used the linear structures as a convenient working model. In contrast, Willstätter had eliminated other possible structures through careful analysis and fully believed in his linear model. In addition, although these models were presented as linear octomers, Willstätter made it clear that this was the minimum length necessary to explain the presented results and the actual materials could certainly be larger, thus paving the way for the eventual understanding of these materials as macromolecules. As such, Willstatter certainly deserves greater recognition for his important contributions to our modern understanding of polyaniline materials.

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Historical Article

Path to the Synthesis of Polyacetylene Films with Metallic Luster: In Response to Rasmussen's Article

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Abstract. The 2000 Nobel Prize in Chemistry was awarded jointly to Alan J. Heeger, Alan G. MacDiarmid, and Hideki Shirakawa "for the discovery and development of conductive polymers." Unlike metals, organic polymers or plastics do not conduct electricity. The three laureates found that polyacetylene can be doped on a film, which was initially synthesized by Shirakawa following a failed experimental trial by a Korean scientist, Hyung Chick Pyun. Later, Pyun insisted that he was the discoverer of polyacetylene films with silvery sheen. This note sheds light on the true history of the synthesis of polyacetylene films.

Keywords: polyacetylene film, Nobel Prize 2000, polymerization, Ziegler-Natta catalyst, fortuitous error, serendipity.

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INTRODUCTION

On October 10, 2000, the Royal Swedish Academy of Sciences announced that plastic can indeed, under certain circumstances, be made to behave like a metal – a discovery for which Professor Alan J. Heeger, Professor Alan G. MacDiarmid, and I, Hideki Shirakawa, received the Nobel Prize in Chemistry in 2000. This public announcement was followed by a story about the initial discovery of polyacetylene films: "once – by mistake – a thousand-fold too much catalyst was added."[1] Professor Bengt Nordén, chairman of the Nobel Committee for Chemistry, also used the term "mistake" in the prize announcement.[2] Commenting on a trigger of our achievements, he stated that the road to the discovery had started in 1967, when Shirakawa found that "by mistake" "a student [Pyun] had taken a thousand times too much catalyst" when polymerizing acetylene gas to make something called polyacetylene. At the beginning of my Nobel lecture entitled "The Discovery of Polyacetylene Film: The Dawning of an Era of Conducting Polymers," given on December 8, 2000 at Aula Magna, Stockholm University, I acknowledged Dr. Hyung Chick Pyun (1926–2018), among other important contributors, for sharing a "fortuitous error" that motivated further work toward the discovery of polyacetylene films.[6]

In his recent article,[3] Professor Seth C. Rasmussen discussed the event leading to the discovery of polyacetylene films by fortuitous error, based mainly on a "working English translation" of Pyun's account written in Korean. Carefully reading Pyun's two original documents written in 2002 and 2013 (through Japanese translation), [4,5] I found numerous factual errors, distortions, and assumptions. In this note, I would like to correct them and present what happened regarding the synthesis of polyacetylene films with metallic luster, based on my memory and my laboratory notebooks.

PYUN'S EXPERIMENTAL TRIAL

In his article,[3] Rasmussen asserts that the core of the discovery of polyacetylene films resulted from the performance of both myself and Pyun, a scientist visiting Japan from South Korea, quoting the "fortuitous error" that I mentioned in the Nobel Prize lecture[6] and is described in the *Les Prix Nobel: The Nobel Prizes 2000* and its reprints.[7] This description is acceptable if the core of the discovery is interpreted as an important trigger for the synthesis of the metallic polyacetylene films. I repeat that the occurrence of the "fortuitous error" was a trigger for the subsequent synthesis of metallic polyacetylene films but not the successful synthesis itself.

However, Rasmussen does not present the discovery in this manner. Most descriptions in the article are based on Pyun's accounts[5] and third-party records, such as press reports by the Nobel Foundation and Royal Swedish Academy of Sciences at the time of the Nobel Prize announcement and award. The article lacks any of my own input, except for my printed Nobel Lecture and scientific papers. As a result, it depicts a biased account that contains serious errors.

In his document,[5] Pyun insists that he was an actual discoverer of polyacetylene films and not Shirakawa. He states, "A good result will be anticipated with the stirring speed reducing as much as possible during the polymerization process. One day, when I tried an experiment on acetylene polymerization with this idea, it happened that a stirring motor stopped suddenly, presumably due to setting the rotation speed too slow. I was very panicked at first, but with careful inspection of the reaction flask, I found that a silvery membrane had formed on a surface of the catalyst solution, to my surprise." He adds, "On this day, for some reason, the then assistant [Research Associate] Shirakawa was not in the laboratory."

These descriptions contain the two following factual errors: 1) "a silvery membrane had formed" and 2) "On this day, for some reason, the then assistant Shirakawa was not in the laboratory." The exact date of "this day" is not clear because the trial run was not recorded on my laboratory notebook as the trial run was not mine. According to my memory, what happened on "this day" is as follows. One of Pyun's two academic supervisors was Professor Yoneho Tabata (1928–2019) of Tokyo University, Faculty of Nuclear Engineering. Due to Tabata's prolonged trip to the United States, Pyun could not perform any primary research during the period, which he described as his free time. The "this day" that Pyun refers to likely occurred in August 1967 considering these circumstances. One day, Pyun came to my office and asked to try acetylene polymerization as he was interested in acetylene polymerization and polyacetylene. I provided an experimental protocol for performing acetylene polymerization as a trial experiment at my research laboratory (room number 404) at the Research Laboratory for Resources Utilization, Tokyo Institute of Technology.

As mentioned above, because this trial run was not my own research work, any relevant data, including the date and conditions for the polymerization, were not recorded in my laboratory notebook. I gave Pyun normal guidelines for the polymerization process, such as a solvent, species of the catalyst components, and their concentrations. I expected that the product would be in a powdered form as usual. After briefly instructing Pyun on the handling of lab wares and equipment, I returned to my office, a preparatory room next to the No. 404 lab.

The No. 404 lab, which I used as my own lab, was designed by Professor Sakuji Ikeda (1920–1984) as a tracer experiment for handling radioisotopes. When you enter the room from the passageway, you enter my office, a small preparatory room that students and I used as a study room. The No. 404 lab is accessible from the preparatory room. According to the lab rule, study desks were not placed in the No. 404 lab, so researchers and students spent their time in the preparatory room and only entered the lab to perform experimental work. At that time, the lab was freely open only to graduate and undergraduate students who were under my guidance. Therefore, Pyun could not carry out experiments for acetylene polymerization in my absence.

On that day, after some time, Pyun came to my office to say that the polymerization had stopped. As this experiment should have been easy to perform, I was puzzled by what could have gone wrong. I checked the apparatus carefully with him, the occurrence being inconceivable. I then found that the magnetic stirrer had stopped and, after watching for a while, the mercury manometer, which indicates the acetylene's pressure, did not decrease.

We confirmed that the acetylene monomer had not polymerized, meaning that the reaction had not taken place. The reaction flask was, therefore, detached from the vacuum line, and we looked carefully inside the flask. If the reaction had proceeded normally, we would have observed a powdery product, but instead, we found that a black flappy or spongy matter had formed on the surface of the catalyst solution. The product, which was extracted using a pair of tweezers, was like a black rag. It was not "the silvery membrane" that Pyun described. If the reaction had proceeded normally, the product would have been a powder. The trial was clearly a failure, and Pyun was in agreement. I remember the result of the run clearly, even now.

SUCCESSFUL SYNTHESIS OF POLYACETYLENE FILM WITH METALLIC LUSTER

I still do not fully understand why Pyun's trial run failed. However, because the product was not a powder but a quite different form, I thought that it might be possible to synthesize polyacetylene into a thin film by changing the polymerization conditions. I was also strongly motivated to clarify the cause of the failure. Immediately after Pyun's failure, I started a series of polymerization experiments with graduate students under my guidance by changing the concentration of the catalysts and other conditions. Pyun was not involved in this series of experiments.

Within one to two weeks, my students and I could successfully synthesize films of polyacetylene that were self-standing and could be easily handled by increasing the concentration of the catalysts by 1,000 or more times the normal condition. The self-standing film was such that it did not need lining. By improving the polymerization conditions and methods, we also found that a thin film with a silvery metallic sheen could be obtained by polymerizing acetylene on the glass surface of a reaction flask when a concentrated catalyst solution was applied to the glass surface of the flask. The side stuck to the glass surface of the reaction flask displayed a metallic luster, while the other side facing the acetylene gas took on a black color and a matted texture.

The difference in the appearance of the two sides was clarified by observations made through transmission and scanning electron microscopic observations. Scanning electron microscopic observations of a surface of the polyacetylene film revealed that the film was composed of entangled fiber-like long microcrystals (fibrils) with a diameter of ca. 200 Å. The morphology of an extremely thin film with several micrometers in thickness observed by a transmission electron microscope was also found to have the same microstructure. From these observations, it was clear that the whole film was composed of entangled fibrils.

Because these films are composed of loosely entangled fibrils and inevitably lead to low bulk density, the incident light on the surface of the fibrils scattered randomly. Consequently, both surfaces of these films appear matted black due to random reflections. The films formed on an interfacial surface of vapor/liquid (catalyst solution) reflect the trend strongly. Therefore, both sides of such films are the same matted black and show no metallic silverly luster. However, on the side of the film that touches the glass wall of the reaction flask applied by the catalyst layer, the growing fibrils are forced onto the glass wall during the polymerization reaction. Thus, the surface of the film facing the glass wall becomes flat with a higher density than the other surface. When incident light shines on this side of the film, the light reflects in the same direction, and the surface presents a silverly metallic sheen. Following this process, *cis*-polyacetylene synthesized at a very low temperature (as low as -78 degrees centigrade) has a copper-like reflection color. All double bonds in the *cis*-polyacetylene have a *cis* form of geometric isomeric double bonds through the conjugated molecular chain. The *cis* form of polyacetylene results in a reflection spectrum shift to higher energy than that of *trans* form, because the bandgap of the *cis* form is larger than the *trans* form, which has a silvery sheen.

THE FORTUITOUS ERROR

I referred to Pyun's trial run as a "fortuitous error" because, as a result of that failure, a black flappy or spongy matter was formed instead of the powdery product that should have formed under normal conditions. This accident became a trigger for the successful synthesis of films with a metallic sheen. Immediately after Pyun's trial run, while continuing experimental trials to clarify the reason for the failure, we found that films formed on the surface of a much more concentrated catalyst solution – a thousand times more – instead of the traditional mmol/liter order of concentration. Using these films as a specimen for infrared spectroscopy, Raman scattering spectroscopy, X-ray diffraction, and normal vibrational analysis, among other tests, our primary intention to clarify the mechanism of acetylene polymerization reactions with Ziegler-Natta catalysts was accomplished within an unexpectedly short period (just less than two years). Because the unforeseen failure of Pyun's test run had triggered the successful syn-

theses of polyacetylene films, and I thought of this fortuitous error as a result of "serendipity," I introduced this occurrence as a typical example of fortuitous error at domestic academic meetings and workshops, as well as international conferences and institutions. However, our polyacetylene study, published in academic journals internationally or domestically received little attention at the time.[8]

As context, it may be pointed out that research activities on organic and polymeric semiconductors declined globally after the 1960s, after being very active from the early 1950s to the 1960s. In particular, Natta et al. succeeded in synthesizing polyacetylene by the polymerization of acetylene using so-called Ziegler-Natta catalysts in 1958.[9] The product was an intractable black powder used for the elucidation of various chemical and physical properties of polyacetylene.

Several years after our successful synthesis of polyacetylene films, I was invited to conduct collaborative research with MacDiarmid, Department of Chemistry, University of Pennsylvania. MacDiarmid had been intrigued by the silvery metallic luster of the polyacetylene film (with a silvery sheen) when he visited and gave a seminar at the Tokyo Institute of Technology in 1975. In September 1976, we began joint research on the chemical and physical properties of polyacetylene with Heeger, a solid-state physicist of the Physics Department at the University of Pennsylvania. On November 23, 1976, when we tried to add a small amount of bromine to a piece of polyacetylene film, we found, to our surprise, that the electrical conductivity of the polyacetylene film increased a hundred thousand times. This epoch-making result was reported at an international conference held in New York City in May 1977, and we followed up with two consecutive communications.[11] The result initiated considerable interest among many researchers around the world. The newborn field was named conductive (conducting) polymers and synthetic metals, and a new journal entitled *Synthetic Metals* was born in October 1979. With increasing research activity in this field, the episode of the successful synthesis of the silvery films of polyacetylene was shared widely among researchers.

When I delivered talks at academic meetings and lectures at universities and institutions, I referred to the episode as a typical serendipitous event. As Pyun's trial run was unsuccessful, I did not mention his name in view of his honor. To avoid embarrassing him, I referred to him in evasive terms, such as "a foreign researcher," "a visiting researcher," and sometimes simply "a researcher," "a student," or "a graduate student." I now regret not mentioning his name. The episode resulted in gossip among researchers about how this "foreign researcher" did not understand Japanese and could not follow directions. I am very sorry that Pyun had to endure this.

PYUN'S RESEARCH SUBJECT

I do not remember the exact date of Pyun's visit as a foreign researcher to the lab of Ikeda, Division of Macromolecular Materials, Research Laboratories for Resources Utilization, Tokyo Institute of Technology, as I have no written record of his visit. However, his visit occurred one year after I started my post as a research associate in the division in April 1966.

According to Pyun's document,[5] he visited Japan in May 1967 and returned to Korea in March 1968. I am unsure when I first met Pyun in person or at laboratory meeting. However, I have a vivid memory of how he first introduced himself as he told me that his family name was "HEN 邊" – that is, the side (hen) of a triangle. I knew that he grew up in the era of Japanese colonization, and he was forced to be educated in Japanese. Later, he struggled through the Korean War (1950–1953). However, he never communicated about his difficult life in Korea. He was always easy to work with and he never expressed any inability to understand Japanese.

As I had only recently joined the lab, I was not informed about how and why Pyun had joined the Ikeda lab as a foreign associate. However, I knew vaguely that his research purpose was the clarification of certain polymerization mechanisms using isotopes as one of his supervisors was Tabata, Department of Atomic Engineering, University of Tokyo. I found out only recently that, as mentioned above, Pyun's research in Tabata's lab was interrupted for some time because Tabata's return from his visit to the United States was delayed. During this period, Pyun visited my lab to conduct the trial run on the polymerization of acetylene, feeling that he had free time to work on subjects outside his main focus during Tabata's absence.

According to Rasmussen's article and Pyun's documents, Pyun's research subject was the copolymerization of ethylene and tetrafluoroethylene and the analysis of its molecular structure by infrared spectroscopy. There was no discussion at the lab meeting that Pyun had joined the research group to study acetylene polymerization. At that time, I was unaware of whether Ikeda was unwilling for Pyun to deviate from his primary subject of interest, and I did not know that Tabata's return had been delayed. However, I accepted his offer to perform a trial run without hesitation. It was very clear that the

trial run was an experience test for him, and he did not undertake it as a research collaborator in the ongoing work in Ikeda's lab.

It turns out that, as described below, Pyun conducted two or more related tests after this initial test. However, I am sure that his additional experiments were performed after our group's establishment of the synthesis of self-standing polyacetylene films with a silvery luster. I was hired as a research associate in the Division of Macromolecular Materials, Research Laboratory for Resources Utilization, Tokyo Institute of Technology, on April 1, 1966, and I continued my research work in the same lab for five years during my graduate student days. At that time, Dr. Shu Kambara (1906-1999) was the professor, and Ikeda was the associate professor of the division. Soon after, Kambara retired on March 31, 1967, and subsequently, Ikeda was promoted to the position of professor on August 1, 1967.

Ikeda had been engaged in research on the vulcanizing mechanism of natural and synthetic rubber using radio isotopes from his associate professor days. After becoming professor of the division, he then continued to work on the mechanism of ethylene and acetylene polymerization with Ziegler-Natta catalysts using tracer techniques. When I became a research associate, Ikeda was very close to his work on ethylene polymerization and had just started research on acetylene polymerization, and I participated in this work.

When Pyun came to my lab to request permission to perform a trial run on acetylene polymerization, his intention, I later realized, was quite different from what he originally stated. When I was a graduate student, Dr. Masahiro Hatano (1930–, who later moved to the Chemical Research Institute of Non-Aqueous Solutions, Tohoku University) was a research associate under Kambara and undertook research on the polymerization of compounds with carbon-carbon and carbon-nitrogen triple bonds for the synthesis of polymer semiconductors. The Kambara and Ikeda Lab has a long history of research on acetylene polymerization and polyacetylene, and Hatano developed acetylene polymerization for many years and accumulated relevant techniques.

Pyun was interested in related research work on semiconducting polymers, which he sought to learn more about by joining Ikeda's group. In fact, he wrote in his document[5] that "I thought of spending my unexpected free time carrying out acetylene polymerization as I learned from various reports in the lab that this lab [the Kambara and Ikeda Lab] had conducted related studies for nearly ten years, and I was much interested in such work." Regarding his research purpose, he also writes, "I thought that even if acetylene polymers are powder, if the polymerization can be controlled to produce particles larger in size, various properties [of acetylene polymers], such as conductivity, would be closer to true values." As mentioned above, he writes that it would be better to decrease the stirring speeds as much as possible during the polymerization reaction to increase the size of the particles. I never heard such a clear idea from him at that time and only learned of it after reading his document. It is worth noting that while he writes that it would be better to increase the size of particles, he provides no idea of how to produce polyacetylene in film form.

As mentioned above, Pyun's trial run was not recorded in my laboratory notebook. While it would be best to check Pyun's notebook regarding the trial run, I learned from reading his documents that he was unable to bring the notebook home following Ikeda's instructions. His laboratory notebook went missing after that.

His document[5] include many inconsistent descriptions of his stay in Japan. The following is one example: "About two weeks after starting my work at Professor's Tabata lab at the University of Tokyo, I went to the Tokyo Institute of Technology to take the deuterated stocked ethylene in the Ikeda lab. There I encountered Professor Kambara's retirement memorial lecture, which had started just then in the auditorium." He continued with the following observations: "The audience was full in the auditorium, and the title was 'A History of the Development of Polymer Science and Engineering in Japan.' I was staggered on hearing Professor Kambara's words in the last part of his lecture in front of such a large audience. He disclosed 'a long-awaited method of producing polyacetylene in the form of polyacetylene film had been attained in Japan, and the inventor is the assistant [Research Associate] Shirakawa of the Ikeda lab.'"

Kambara's retirement date was March 31, 1967, and by custom, lectures by retiring professors were held just before their retirement. If the date of Pyun's visit to Japan was May 1967, as noted in Rasmussen's article, it would be impossible for him to attend Kambara's final lecture as it was before his visit to Japan. Therefore, this must be a continuity error.

Pyun further infers that the abovementioned audiences provided information to the mass media in Japan and that the media caused a media circus immediately after the announcement of Shirakawa's Nobel Prize in Chemistry. Soon after the announcement by the Nobel Committee on October 10, 2000, three young journalists visited my home and questioned me closely as to why Pyun was not a joint prize winner, which left me with an unpleasant memory. The journalists were from one of three big presses in Korea; if my memory is correct, the press was *The Dong-a Ilbo*. From this, I learned for

the first time that Pyun was known as a leading scientist in the synthesis of polyacetylene in Korea, and thus he missed receiving what would have been Korea's first Nobel Prize in Chemistry.

I have no memory of any discussion with Pyun regarding research, especially on acetylene polymerization and the properties of polyacetylene during his one-year stay at the Tokyo Institute of Technology and the University of Tokyo. However, I realized that Pyun was highly interested in polyacetylene as an important candidate in semiconducting polymer by reading related articles before visiting Japan. In his document, he describes that while in Japan "my study of polyacetylene study was stopped after all…," calling up for me his strong emotion toward polyacetylene study.

RECORD OF PYUN'S WORK IN MY LABORATORY NOTEBOOKS

To refresh my memory to write this note, I checked my 15 laboratory notebooks used during my period at the Tokyo Institute of Technology and more than 20 notebooks related to my research works. These showed me that Pyun conducted several experiments on acetylene polymerization. The date of these experiments cannot be specified, but presumably, it was in mid-September 1967, after we established the synthetic method of self-standing polyacetylene films with a silvery sheen.

The first assumable description was a series of runs in which Pyun carried out three polymerizations of deuterated acetylene by changing the polymerization temperature. The results of the elemental analyses of the deuterated polyacetylenes are recorded in my notebook No. 3, page 442, as Pe-1 polymerization temperature 60 degrees centigrade, Pe-2 polymerization temperature 20 degrees centigrade, and Pe-3 polymerization temperature -78 degrees centigrade, respectively. The code "Pe" can be understood as Pyun's initial. He synthesized deuterated acetylene by himself as precursors for the synthesis of deuterated ethylene, such as $C_2H_2D_2$ and C_2D_4, as monomers for deuterated polyethylene. It was my assumption that he tried to polymerize the deuterated acetylene prior to his primary purpose.

The next record was on the mass-spectroscopy analysis of ethylene-d_4, synthesized by Pyun. The result is described in my notebook No. 3, page 444, October 13, 1967. The ethylene-d_4 was synthesized by an additional reaction of deuteron to acetylene-d_2 for the copolymerization of ethylene and tetrafluoroethylene. For his primary purpose, Pyun synthesized acetylene-d_2 himself as an intermediate for the synthesis of ethylene-d_4. Presumably, he attempted to polymerize the acetylene-d_2 as a monomer.

His experimental results, mentioned above, were recorded twice in my notebook because I was familiar with the application procedures for elemental analysis and mass-spectroscopy. Thus, his products, acetylene-d_2 and ethylene-d_4, were passed to each analytical lab via me, and the data were, necessarily, recorded in my notebook.

The first appearance of the term "polyacetylene film" is recorded clearly in my notebook No. 3, page 452, on November 8, 1967, as X-ray scattering analysis was carried out in the X-ray analysis room of the Department of Textile Engineering. The measurements were performed at two different temperatures – 21 and 80 degrees centigrade – to measure the estimated thermal expansion coefficient of polyacetylene films. It should be noted that these analyses were carried out for a series of measurements of the various physical properties of polyacetylene films, and there was no relation to Pyun's research.

CLOSING REMARKS

The Nobel Committee announced that our Nobel Prize in Chemistry in 2000 was awarded "for the discovery and development of conductive polymers" based on the doping of polyacetylene attained by the three laureates. It should be noted that it was not for the synthesis of polyacetylene films. However, there is no doubt that the synthesis of polyacetylene films was a key factor in the discovery of conducting polymers. In this account, I have presented the truth to the best of my ability, based upon my memories and my notebooks. I further want to stress that Pyun's contribution was minimal and that his claims that he was the discoverer of the synthesis of polyacetylene were his strong belief, but they were not supported by the truth of what happened. However, his one-year stay in Japan was not satisfactory, and as one of the concerned personnel, I regret that his research work was not attained fully due to several unfavorable events during his stay in Japan.

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Historical Article

Comments on Shirakawa's Response

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As both an active researcher in the synthesis of conjugated materials and a chemist-historian that has spent the last decade attempting to detail and clarify the history of conjugated and conducting polymers,[1,2] I am overjoyed that Prof. Shirakawa has elected to provide additional personal details relating to the discovery and development of polyacetylene films. Shirakawa has provided this material in response to my most recent *Substantia* paper that details newly revealed accounts by Hyung Chick Pyun (1926-2018), who was a visiting Korean scientist that carried out the initial experiment that led to these films.[3] This material is critical to advance our understanding of this important historical event. At the same time, however, I was quite disappointed to find that Shirakawa viewed my paper as biased, particularly as this puts me in the unpleasant position of having to defend my integrity as a chemist-historian. I approach my historical efforts with great care and the integrity of these efforts is something that I take quite seriously.

According to Merriam-Webster, when used as a verb (as the case here), bias means "*to give a settled and often prejudiced outlook to*". In a more general way, bias typical refers to an emphasis in favor of or against an idea or entity, usually in a manner that is closed-minded, prejudicial, or unfair. Of course, the goal and expectation within history is that descriptions of historical subjects, general interpretations of the past, and historical explanations are fair and not misleading. This does not mean that bias does not occur within history and the historian C. Behan McCullagh describes four common ways in which historical writing can be biased.[4] Still, McCullagh goes on to explain that such cases are only biased if they occur because the historian wants a particular outcome, normally to further certain personal interests. Of course, one can describe Pyun's account as biased, which would be valid. After all, it is a personal account with significant self-interest, as are many such personal accounts, and was written by a man who felt grievously wronged. Still, the use of such sources does not necessarily make the resulting historical analysis biased. Historians have long been aware that written documents reflect the concepts and interests of their authors.[4] This issue is generally dealt with by not taking material at its face value, but to construct explanations of its origins that will account for its features as much as possible, after which efforts are made to find coherence among the various expla-

nations to decide what really happened. This is precisely the approach taken in the analysis of Pyun's account and its incorporation into a larger view of the discovery of polyacetylene films, including highlighting aspects that were known to be inaccurate.

Shirakawa is also critical of the sources used in the analysis and presentation of the discovery of polyacetylene films, stating "*Most descriptions in the article are based on Pyun's accounts and third-party records, such as press reports by the Nobel Foundation and Royal Swedish Academy of Sciences at the time of the Nobel Prize announcement and award. The article lacks any of my own input, except for my printed Nobel Lecture and scientific papers.*" This, however, is a misrepresentation of the sources used. While third-party sources were indeed used, this was only in discussion of how the event has been commonly portrayed by others, as well as highlighting errors in many of those descriptions of the event. In terms of constructing a more accurate narrative of the discovery of polyacetylene films, the primary sources beyond Pyun's account were Shirakawa's scientific publications, his published Nobel Lecture,[5] his Nobel autobiography,[6] and a reflection by Shirakawa on the polyacetylene film synthesis that was published in the *Journal of Polymer Science: Part A. Polymer Chemistry* in 1996.[7] All of these sources were written by Shirakawa and include his personal descriptions of various aspects of the event. As such, the published narrative included all available sources at the time. Of course, as I pointed out in the Substantia paper:[3] "*the truth is Shirakawa has actually said very little on the subject and what has been said is somewhat vague.*" While Shirakawa is now sharing additional material that will further add to our understanding of these events, the previous work cannot be criticized for not including details that had never been communicated.

I look forward to a deeper study of this new account from Shirakawa, which will likely change our view of the details of this event. The addition of new sources is a common aspect of historical study, which can often result in refinement, correction, or even drastic re-evaluation of historical events. As with the previous account of Pyun, this will require analysis of Shirakawa's newly presented account and renewed efforts to find consistency between all of the available sources to decide what really happened. Clearly, this will require more significant time and effort than what I have been able to dedicate for the preparation of this short commentary. However, initial review seems that Shirakawa is now implying that Pyun's initial experiment did not produce polyacetylene films, but only "*a black flappy or spongy matter*". This failed experiment then served as motivation for further experiments by Shirakawa and his students, which ultimately resulted in the successful generation of polyacetylene films. As emphasized by Shirakawa, Pyun was not involved with these additional experiments and stated that "*Pyun's contribution was minimal*". This new narrative, however, does not seem to be consistent with multiple statements Shirakawa has made in the past. Such statements include an acknowledgement "*to Messrs. H. C. Pyun and T. Ito for the preparation of poly(acetylene) films*" in his 1971 paper,[8] as well as the following statement from his autobiography that describes Pyun's initial product as a film:[6] "*when a visiting scientist tried to make polyacetylene in the usual way, he only produced some ragged pieces of a film.*" And then, there is of course the acknowledgement to Pyun made in his Nobel lecture:[5] "*...and to Dr. Hyung Chick Pyun with whom I encountered the discovery of polyacetylene film by the fortuitous error.*" Finally, I must point out that Shirakawa makes various statements concerning the history of organic semiconducting materials that are not supported by our current understanding of the historical record. Rather than enumerate these specific points, I will just encourage the reader to consult my extensive work on this topic for the most current analysis of this history, as well as discussion of the associated historical record.[1,2]

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Historical Article

Lipids, Chloroform, and Their Intertwined Histories

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Abstract. Lipids and their fatty acid constituents, in particular, have been the subject of academic and industrial research initiatives since their isolation by Michel-Eugène Chevreul in 1813. Fatty acids can be saturated or unsaturated, their physical properties depending on the aliphatic chain length and degree of saturation. They constitute the building blocks of many lipid groups like triglycerides and phospholipids; are key additives in commercial foods, pharmaceuticals, and cosmetics; and can cross cell membranes. Chloroform was synthesized in 1831 by Samuel Guthrie and has had a tortuous history of interactions with mankind: from an anesthetic in obstetrics, dentistry, and surgery, to being labeled as a potential carcinogen in the 1970s. It has also had important nonmedical applications such as in chemical engineering mass transfer systems designed to estimate binary gas diffusion coefficients. Although chemically dissimilar, lipids and chloroform intertwined their scientific paths through the work of Jordi Folch and associates in the 1940s-1950s, in which many lipid-based brain molecules were isolated and characterized. This article outlines the separate histories of lipids and chloroform, and those research initiatives in which they have acted synergistically. The narrative covers the interplay of chemical compounds with different historical backgrounds, but with physical properties which continue to foster their interaction.

Keywords: lipids, fatty acids, cell membrane structure and function, chloroform synthesis and uses, lipid-based brain tissue components.

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I. INTRODUCTION

Modern-day students and young researchers sometimes fail to identify events in one discipline that can help explain similar phenomena in another. They also have difficulty envisioning how materials with different physicochemical properties can lead to common applications. It is precisely this latter problem that serves as the goal of the present article. Lipids and chloroform have long, separate histories. They have also shared scientific pathways for many decades, a fact likely unknown to most readers.

As the initial step of an experimental research project involving a specific class of lipids and chloroform, their individual histories were scrutinized. Their isolation, characterization, and synthesis were thoroughly studied, in many cases going back to the original published sources in the 19th Century.

It is our goal to summarize the historical scientific highlights of lipids (fatty acids in particular) and chloroform, leading to their combined usage to this day. Their individual histories will be addressed separately, while their intertwined pathways will be covered in the last section. The best example of the synergistic use of lipids and chloroform is that their physical and chemical properties served to determine the structure and function of new families of mammalian brain tissue components.

II. LIPIDS AND THEIR FATTY ACID CONSTITUENTS

Biochemistry is the science dealing with the chemistry of life, as well as the title of a timeless textbook written by Professor Albert L. Lehninger of The Johns Hopkins University School of Medicine, Baltimore, MD, USA.[1] A chapter of this venerable reference is entitled "Lipids, Lipoproteins, and Membranes", a subject matter relevant to this work. Lipids are natural substances present in animal and plant tissues. They are mostly insoluble in water, but soluble in many organic solvents. Lipids are actually families of compounds, with similar physical and chemical characteristics, and can be conveniently grouped according to their backbone structure (Table 1 and Figure 1). For example, acylglycerols (triglycerides) are the most abundant lipids in nature. The reader must have heard of them when, during a routine medical checkup, the doctor pulled his/her ear for having a high blood triglyceride level. Solid triglycerides are known as "fats" and their liquid counterparts as "oils". They all have a glycerol (triol) backbone joined through ester linkages to fatty acids. The latter consist of a hydrocarbon (aliphatic) chain of varying length and degree of saturation, indicating the presence or absence of double bonds, with a carboxylic acid terminus. The following discussion will focus on fatty acids, since they are the building blocks of many lipid groups, as well as being part of our current research interests. In a later section, we will return to the broader lipid family as we explore its historical relationship with chloroform.

Fatty acids were first isolated in 1813 from animal fats ("corps gras") by the French scientist Michel-Eugène Chevreul (1786-1889), Professor of Chemistry at the Lycée Charlemagne, Paris.[2] They have been the subject of extensive academic and industrial research ever since. Within the body, fatty acids are found in their esterified form since they are practically insoluble in water. To illustrate with a numerical example, the saturated 6-carbon hexanoic acid has an approximate solubility of 1 g fatty acid per 100 g water at room temperature. This translates to a very small fatty acid mole fraction of order 10^{-3}, with this quantity being relevant in mass transfer processes such as those found typically in the field of chemical engineering. The water solubility decreases considerably as the number of aliphatic carbons in the chain increases. Fatty acids are transported in blood bound to serum albumin, a globular protein with an approximate molecular mass of 68000.[1] They can also cross cell membranes, of which they are key constituents, by diffusion (proportional to a concentration gradient) and protein-facilitated mechanisms.[3-12]

Fatty acids are metabolic precursors of many physiologically-relevant molecules as well as a source of energy for the organism. Free (unbound) fatty acids are either unsaturated (single or multiple double bonds along the aliphatic chain) or saturated (no double bonds). They are named according to the number of carbon atoms in the chain and the location of the double bonds, if any. For example, the 16:0 saturated fatty acid corresponds to the 16-carbon palmitic acid [$CH_3(CH_2)_{14}COOH$] which melts at +63.1°C. On the other hand, the 16:1(D9) monounsaturated fatty acid represents a 16-carbon chain with a double bond between the 9 and 10 carbons (by convention the carboxylic acid is the first carbon). This compound is known as palmitoleic acid

Table 1. Lipid classification according to their backbone structure.[1] The saponifiable lipids can be hydrolyzed to their building blocks which include fatty acids, while the nonsaponifiable lipids cannot be hydrolyzed. Triglycerides are the most numerous in nature. Phospholipids are the main constituents of cell membranes.

	Lipid Type	Molecular Backbone
complex (saponifiable)	acylglycerols or triglycerides	glycerol
	phosphoglycerides or phospholipids	glycerol 3-phosphate
	sphingolipids	sphingosine
	waxes	high-molecular-weight nonpolar alcohols
simple (nonsaponifiable)	terpenes	multiples of the 5-carbon hydrocarbon isoprene
	steroids	perhydrocyclopentanophenanthrene
	prostaglandins	obtained by cyclization of 20-carbon unsaturated fatty acids such as arachidonic acid

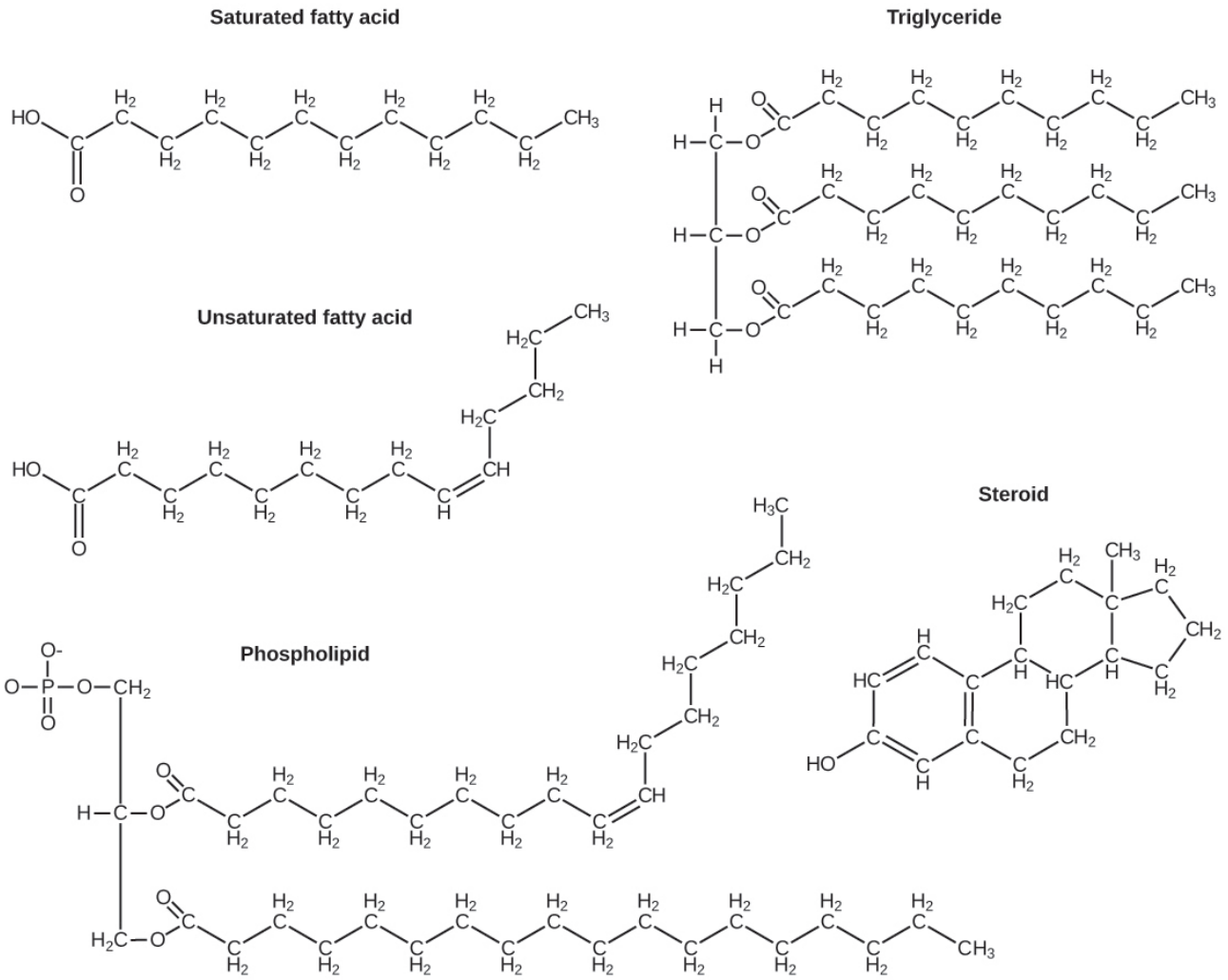

Figure 1. Two-dimensional chemical structures of some of the lipid groups in Table 1. The fatty acids are the building blocks of triglycerides and phospholipids, which are saponifiable through alkali hydrolysis. The simple lipids like steroids are nonsaponifiable. Source: https://cnx.org/resources/00a0b827644d73bfb8695b81a7c7801a.

$[CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH]$ which melts at -0.5°C. Industrial applications of fatty acids include the preparation of soaps, detergents, and lubricants. They are also used as additives in foods, cosmetics, and pharmaceuticals. The book *Fatty Acids and Their Derivatives* by Anderson W. Ralston, Assistant Director of Research at Armour and Company, Chicago, IL, USA, is still the classic starting point for both researchers and enthusiasts of fatty acid biochemistry.[13]

Due to their importance in physiology and food science, fatty acids have been extensively characterized from the standpoint of their physicochemical and dissociation properties. The chronological development of some of these research lines is as follows: a) their very low aqueous dissociation constants were determined as far back as the 1930s, indicating that the vast majority of the fatty acid molecules in solution are nonionic;[14-17] b) the mutual solubility of fatty acids and water has been known since the 1940s, demonstrating the effect of aliphatic chain length and temperature on this property;[18-25] c) solute partitioning studies of fatty acids in organic solvent/aqueous systems, relevant to industrial separation and purification processes, have been undertaken since the 1950s to determine their relative solubility in immiscible media;[26,27] d) chemical reactions at oil/water interfaces involving fatty acid-containing molecules were identified in the 1960s, with applications to the hydrolysis of triglycerides in the intestinal tract and to atmospheric photochemistry;[28,29] and e) Lyman C. Craig and coworkers at the Rockefeller Institute for

Medical Research, New York City, NY, USA, designed in the 1940s-1950s a rotary, multi-stage, liquid-liquid extractor using chemical engineering mass transfer principles to isolate and purify fatty acids from their mixtures, with one benchtop model allowing several thousand quantitative extractions in a few hours of operation![30-34] These landmark historical efforts highlight the importance fatty acids have attained in modern science, particularly their relevance in molecular transport across biological membranes which are discussed below.

Solute exchange across cell membranes is vital to animals and plants. Elegant reviews have addressed the subject of transcellular transport of fatty acids, notwithstanding their limited solubility in aqueous media and the existence of lipid-based membrane resistances.[4-6,12] Debate in the literature lingers on the actual mass transfer mechanisms involved, but it is known that fatty acids are transported with relative ease through triglyceride- and phospholipid-rich cell membranes. To support these findings, the fluid-mosaic model for membrane structure was proposed by Singer and Nicolson in 1972[35] and, even though it has been upgraded conceptually in the last fifty years due to refinements in experimental techniques and instrumentation, remains relevant to this day.[36-48] The original model is sketched in Figure 2 (top) and shows a phospholipid bilayer interspersed with globular (integral) proteins. Membrane phospholipids contain fatty acids attached through ester linkages. Their hydrophilic heads point outward to the aqueous media, while the hydrophobic ends (fatty acids) point inward. The upgraded model depicted in Figure 2 (bottom) indicates that membrane architecture is more complex than originally proposed. Current scientific knowledge describes the membrane as consisting of protein and lipid domains. These are constituted by multiple chemical species that interact dynamically with the cytoskeleton and the extracellular matrix. The most important lines of research related to membrane structure and function since the appearance of the fluid-mosaic model[35] are: a) the isolation and characterization of membrane proteins;[38,47] b) elucidation of membrane molecular signaling and trafficking pathways;[41,42,45-47] c) proof of the existence of membrane protein and lipid domains;[36,37,43-47] d) a description of membrane lateral motion, confinement, and turnover;[38-40,42,43,45-47] e) the roles played by membrane-associated cytoskeletal fences and the extracellular matrix in restricting the lateral diffusion of membrane components;[44,47] and f) characterization of the asymmetry of lipid distribution between the leaflets of the plasma-membrane bilayer.[38,46-48] It is clear from these research thrusts that lipid biochemistry is a continuously evolving discipline, and that it plays a pivotal role in advancing our understanding of cell membrane structure and function.

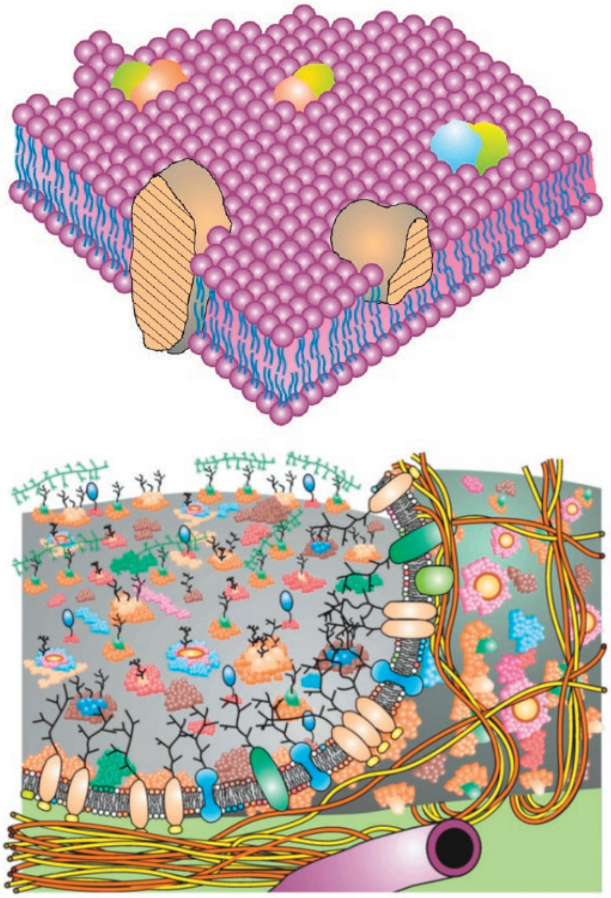

Figure 2. *Top*: Colorized version published by Nicolson (2014)[47] of the Singer and Nicolson (1972)[35] fluid-mosaic membrane model. Originally, it consisted of a phospholipid bilayer with interspersed proteins that could traverse the membrane. The hydrophilic phospholipid heads point to the external and cytoplasmic aqueous media, while the hydrophobic fatty acid tails point inward. *Bottom*: Nicolson (2014)[47] also provided an upgraded membrane model containing what modern-day scientists believe to be more realistic structural and functional features. The basic lipid bilayer architecture is preserved, but in this drawing the membrane has been peeled up (at the right) so that the viewer can appreciate several membrane-associated cytoskeletal (bottom left) and extracellular (top left) interactions. Integral proteins, glycoproteins, lipids, and oligosaccharides are represented by different colors. Protein and lipid domains are evident throughout the membrane. Science moves at a rapid pace in its attempt to unravel the functional attributes of these and many other membrane structures.

We postpone further discussion of lipids to the section following the historical development and uses of chloroform.

III. CHLOROFORM SYNTHESIS AND ITS USES IN MEDICINE AND CHEMICAL ENGINEERING

Chloroform (trichloromethane; $CHCl_3$; CAS Registry Number 67-66-3) was synthesized and purified in 1831 by Samuel Guthrie (1782-1848), who studied medicine under his father's tutelage, and may be considered to be a self-made farmer, chemist, and industrial manufacturer from Sackets Harbor, NY, USA.[49] An interesting historical note is that chloroform's right of discovery was contested for many years, with the French pharmacist Eugène Soubeiran and the German chemist Justus von Liebig claiming priority supported by their independent chemical syntheses. In 1888, a committee appointed by the Chicago Medical Society examined the available scientific evidence and concluded that Guthrie was the rightful discoverer of chloroform, almost sixty years after the events in Sackets Harbor had taken place and forty years after his death.

Guthrie worked in his home/farm laboratory to improve gunpowder preparations (leading to several near-fatal explosions!) as well as carrying out liquor distillations. One of his batch reaction-distillations produced what came to be known as "Guthrie's sweet whiskey", a solution of chloroform in ethanol quite popular among the local alcohol-consuming clientele. The consecutive liquid-phase reactions thought to have taken place in Guthrie's boiler-distiller in his historical synthesis are as follows:[50]

a) Ethanol reacts with calcium hypochlorite to give acetaldehyde, calcium chloride, and water.
$2C_2H_5OH + Ca(OCl)_2 \rightarrow 2CH_3CHO + CaCl_2 + 2H_2O$

b) Acetaldehyde reacts with calcium hypochlorite to give chloral and calcium hydroxide.
$2CH_3CHO + 3Ca(OCl)_2 \rightarrow 2CCl_3CHO + 3Ca(OH)_2$

c) Chloral reacts with calcium hydroxide to give chloroform and calcium formate.
$2CCl_3CHO + Ca(OH)_2 \rightarrow 2\mathbf{CHCl_3} + (H\text{-}COO)_2Ca$

Chloroform has a molecular mass of 119.38 g/mol and is a colorless liquid at room temperature with a sweet, ether-like smell. It has a normal boiling point of 61.2°C, a mass density of 1479 kg/m^3, and a viscosity (a quantity proportional to its resistance to flow) of 5.37 x 10^{-4} Pa×s [kg/(m×s)] at 25°C. These are very important physical properties for describing its behavior in momentum, energy, and mass transport systems such as those typically encountered in chemistry and chemical engineering. For comparison, liquid water has a molecular mass of 18.02 g/mol, a normal boiling point of 100°C, a mass density of ~1000 kg/m^3, and a viscosity of ~1.0 x 10^{-3} Pa×s [kg/(m×s)] at room temperature. Thus, chloroform is denser but less viscous than water at room temperature. Its solubility in water is very low since 1 mL dissolves in about 200 mL water at 25°C. However, as we shall see later, chloroform is an excellent solvent for lipids!

Chloroform was first used as an obstetric anesthetic in 1847 by Sir James Young Simpson (1811-1870) of Edinburgh, Scotland. A very famous patient who received the wonderful chemical on April 7, 1853, was Her Majesty Queen Victoria (1819-1901). After inhaling chloroform for 53 minutes through a folded handkerchief during labor, she delivered her eighth child, Prince Leopold.[50] Illustrations of chloroform's early use as an anesthetic are shown in Figure 3 (top and middle). Following this headline performance, chloroform quickly gained worldwide popularity in related medical specialties such as dentistry and surgery. It has seen action in the treatment of battlefield wounds since the American Civil War [1861-1865; Figure 3 (bottom)]. Readers may also recall its sinister role in well-known fictional crime novels such as Agatha Christie's *The Plymouth Express*[51] and *Why Didn't They Ask Evans?*,[52] in which the assassins subdue their victims temporarily with a chloroform-soaked textile fabric applied to the nose and mouth pending further plans. Due to its potential carcinogenicity, chloroform was banned from human use as an additive in pharmaceuticals and cosmetics by the United States Food and Drug Administration in 1976.[53] However, it is still used in carefully-monitored industrial operations as an intermediate in the production of bactericides, fumigants, insecticides, and fluorinated refrigerants. The synthesis/discovery and medical applications of chloroform have been discussed in excellent books[49,50] and archival references.[54,55]

An important nonmedical use of chloroform, perhaps unknown to the scientific community at large, is its role in mass transfer experiments leading to the estimation of diffusion coefficients (diffusivities) of binary gas pairs, with atmospheric air being the traditional second component. Diffusion coefficients are key parameters in the design and analysis of mass transfer systems typically found in chemical engineering. Examples of the latter include gas absorption, membrane separations, evaporation phenomena, multicomponent distillation, and controlled drug release from diffusion-based systems. The diffusive mass transport rate of a substance in a given medium is directly proportional to its diffusivity; therefore, knowledge of its magnitude is critical to the chemical engineer involved in research or designing an industrial-scale process.[56-59]

Figure 3. *Top*: Inducing chloroform anesthesia by inhalation through a cloth. Source: https://survivalstronghold.com/wp-content/uploads/2016/11/Administering_Chloroform_RAG.png. *Middle*: Chloroform flasks and masks for holding a textile material over the patient's nose and mouth. Source: https://www.riverjunction.com/assets/images/4299/EtherSet.jpg. *Bottom*: Chloroform use for the treatment of a wounded soldier during the American Civil War. Source: https://www.ourgreatamericanheritage.com/wp-content/uploads/2015/08/2363273317_f9cc2da0ea_o.jpg.

The classical method to estimate binary gas diffusivities is the Stefan column developed by Josef Stefan (1835-1893), Professor of Mathematics and Physics at the University of Vienna, during the second half of the 19th Century.[60,61] The original vertical column consisted of a pure liquid phase of volatile species A up to an initial height z_{10}, overlaid by a gas phase containing the evaporated A and a stagnant species B (usually air). All symbols are defined in the Nomenclature. Through the years, the most common operational version of the column, depicted in Figure 4, has been the descending interface modality, which does not require liquid replenishment. In this case, the liquid-gas interface descends to $z_1(t)$ after an elapsed time t following the start of the evaporation-diffusion process, while gas A ascends by diffusion and convection (bulk gas motion) to the top of the column at z_2. A sweeping stream of pure gas B flows steadily and slowly at the top to remove gas A, maintaining its concentration at essentially zero at that location. This boundary condition is critical when developing mathematical models for the transport of gas A within the column. For the experimental situation depicted in Figure 4, the solution of the mass conservation differential equations in the gas and liquid phases is well established in the literature,[58] yielding a simple algebraic expression from which the diffusivity of A in B may be calculated:

$$z_1(t) = z_{10} - (z_2 - z_{10})\left\{\left[1 + \frac{\lambda t}{(z_2 - z_{10})^2}\right]^{1/2} - 1\right\} \quad (1)$$

$$\lambda = 2\frac{c}{c_L} D_{AB} \ln\frac{1 - y_{Az_2}}{1 - y_{Az_1}} \quad (2)$$

In Equation (1), the z-coordinates represent specific vertical locations in the column (m), with z = 0 corresponding to the bottom (refer to Figure 4), and t is time (s). In Equation (2), c is the molar density of an ideal gas at constant temperature and pressure (mol/m^3), c_L is the molar density of pure liquid A (mol/m^3), D_{AB} is the Fickian binary gas diffusivity of A in B (m^2/s), and y_A is the mole fraction of gas A at a specific location within the column. At $z_1(t)$, y_A is calculated by stipulating that vapor-liquid equilibrium conditions prevail at the interface. At z_2, y_A is commonly assumed to be zero due to infinite dilution of gas A in the sweeping stream. For a standard evaporation-diffusion experiment using the Stefan column, D_{AB} can be calculated by regression analysis from Equations (1)-(2) and experimental data of interfacial position versus time, $z_1(t)$.

At this point the alert reader may rightfully ask: How is chloroform related to the Stefan column? Inter-

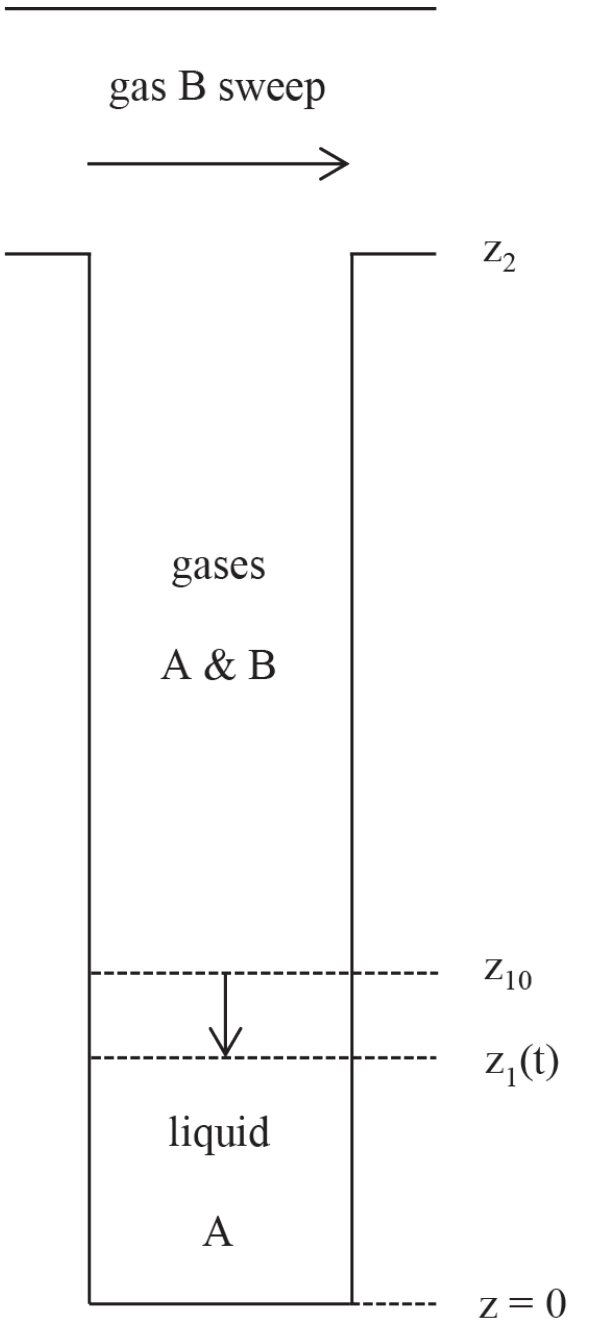

Figure 4. Classic sketch of the Stefan diffusion column showing the liquid and gas compartments.[68,69] The operating modality depicted is that of the descending liquid-gas interface without liquid replacement. Pure volatile liquid A is charged initially to a height z_{10}. Its level falls to $z_1(t)$ after an elapsed time t following the start of the evaporation-diffusion process. Liquid A evaporates at the interface and its vapor ascends through stagnant gas B (air) until it reaches the top of the column at z_2. A steady and slow gas B sweep at the top maintains the concentration of A very close to zero at that location. The binary gas diffusivity of A in B can be calculated from fundamental mass transport principles applied to gas A and experimental interfacial descent versus time data (refer to the text for details). Symbol definitions can be found in the Nomenclature.

estingly, chloroform was one of the first substances tested in the column, when Georg Baumgartner in 1877 determined its diffusion coefficient in air to be about 7.3 x 10^{-6} m^2/s within a temperature range of 17.5-20.3°C.[62] Although this value may seem small at first sight, leading one to the erroneous conclusion that chloroform is a poor diffusant in air, diffusivities of the order 1 x 10^{-5} m^2/s are quite common in binary gaseous systems, as well as being several orders of magnitude *higher* than those in liquids! Therefore, for a given chemical species such as chloroform, its diffusion in gases is very fast relative to its diffusion in liquids. This information is crucial to a chemical engineer when attempting to design a diffusion-based mass transfer system. For the interested reader, well-known compendia are available containing diffusivities obtained by the Stefan column method for many gas pairs, including references to the original publications.[63-65] In addition, a detailed compilation of binary diffusion coefficients for inorganic and organic compounds in air relevant to atmospheric chemistry has been presented recently.[66,67]

In the 1950s researchers first became aware that the Stefan column binary diffusivities may be inaccurate due to "end effects" at the top of the column, where the sweeping stream interacts with the gas phase, and at the liquid-gas interface, where curvature due to surface tension can affect the mass transport area and the diffusion path length for gas A from $z_1(t)$ to z_2. Unfortunately, the early studies could not correlate such end effects to the systems' operational and geometrical settings, limiting their subsequent use. In recent years, we have addressed this gap in scientific knowledge by reporting our efforts to quantify the various Stefan column end effects and their impact on the gas diffusivity estimates. These studies clearly show that factors such as column nonisothermality,[68] sweeping gas stream Reynolds number (dimensionless ratio of fluid inertial to viscous forces) and column aspect ratio (gas phase height to column inside diameter),[69] liquid phase composition,[70,71] and interfacial shape and curvature[72] must be rigorously accounted for in the diffusivity calculations to minimize estimation errors. Our findings to date have hopefully made Stefan column researchers aware of some of the common pitfalls the analyst may find when estimating binary gas diffusivities from experimental interfacial descent versus time data. By continuing this line of research, chloroform and other common volatile solvents may help us attain an even better understanding of the Stefan column mass transport dynamics, leading to more accurate binary diffusivity estimates.

IV. MERGING OF THE LIPID AND CHLOROFORM HISTORIES

Lipids and chloroform intertwined their scientific paths in the 1940s and 1950s through the work of Spanish biochemist/Professor Jordi Folch (1911-1979) and collaborators at the Rockefeller Institute for Medical Research, the McLean Hospital Research Laboratories, and at Harvard Medical School. In 1957, researchers Jordi Folch, M. Lees, and G. H. Sloane Stanley modified their own published procedure for the isolation and purification of total lipids from animal tissues.[73] They developed painstaking analytical methods to extract and purify lipids from animal tissue quantitatively, concentrating on white (axon bundles) and gray (neural cell bodies) brain matter. The team relied on chloroform's dissolving power for organic lipid-based compounds as the key reagent. The tissue was first homogenized with a 2:1 (volume:volume) chloroform-methanol solution followed by filtration. The filtrate, which contained lipid and nonlipid matter, was washed with a five-fold volume of water with minimal lipid losses in the wash. The resulting liquid mixture separated into two phases, the lower chloroform phase containing the total lipid extract. The article qualified the chloroform-based, lipid extraction-purification procedure as: a) operationally simple; b) applicable to any scale of starting biological material; c) capable of decreasing lipid losses incidental to the water washing process; and d) yielding an extract which could be taken to dryness without foaming or splitting of the proteolipids.[73] Besides developing this landmark analytical methodology for extracting total lipids, over the same time period other Folch groups isolated and identified many lipid components of brain tissue, using chloroform both as a solvent and extraction medium.[74-80] Some of the compounds isolated, purified, and characterized from animal brain tissue, with chloroform playing a central role in the extraction-by-dissolution procedures, included: a) α- and β-phosphatidyl serine, phosphatidyl ethanolamine, and diphosphoinositide derived from cephalin (Figure 5); b) strandin consisting of fatty acids and sphingosine; c) proteolipids (lipoproteins) in normal and tumor tissue; and d) extracts of pure lipids. The isolation and characterization of these molecules led the way to elucidating significant aspects of brain tissue biochemistry.

In the last few decades, the lipid-chloroform interaction has continued in applications such as the extraction of lipids from a wide spectrum of animal and plant tissues, as well as in environmental protection and workplace risk minimization by seeking "friendly" alternatives to the solvent's use. Examples of these modern

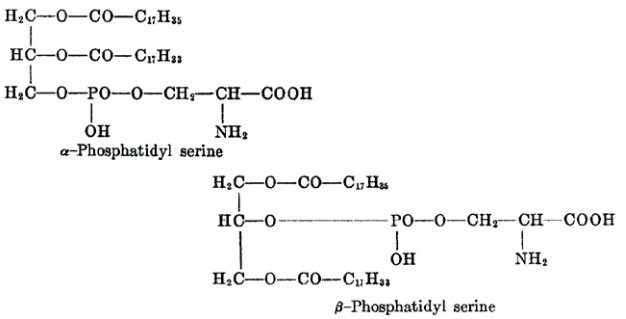

Figure 5. Many lipid derivatives were isolated, purified, and characterized by Professor Jordi Folch and associates from animal brain tissue in the 1940s-1950s using chloroform's dissolving and extraction properties for organic compounds.[73-80] The work of the various Folch teams marked the beginning of the lipid-chloroform scientific interaction, leading to a better understanding of brain tissue biochemistry. It also opened the door to many subsequent research initiatives featuring the synergistic use of both compounds.

research trends involving the synergistic efforts of lipids and chloroform include: a) extraction and purification of unsaturated fish lipids, which require mild treatment to minimize oxidative decomposition and the production of artifacts;[81,82] b) determination of serum triglycerides by extracting the lipids with chloroform, methanol, and diethyl ether, followed by removal of the phospholipids, hydrolysis of the triglycerides, and quantification of their glycerol moieties;[83,84] c) determination of pure lipid solubilities in common laboratory solvents such as chloroform to optimize extraction procedures of diverse biological samples;[85] d) extraction of environmental contaminants such as chlorobiphenyls and other chlorinated pesticides bound and nonbound to fish lipids;[86] and e) reducing the toxicity of chloroform-based tissue lipid extraction methods by substitution with alternate solvents.[87-91] Given their long, intertwined histories in chemistry and biology, it is very likely that lipids and chloroform will continue to find simultaneous use in as yet unforeseen scientific applications.

V. CONCLUSIONS

This article brings together the separate histories of lipids and chloroform. Within the lipid family, fatty acids are highlighted since they are the most abundant in nature and are key constituents of cell membranes. Fatty acids were isolated by Chevreul in 1813[2], and new lipids and lipid-based biomolecules are constantly being identified and characterized due to improved experimental techniques and instrumentation. Not only are lipids of interest to academic researchers, but they find

important industrial use as ingredients in foods, pharmaceuticals, and cosmetics.

Chloroform has gone through its highs and lows along its history. First synthesized by Guthrie in 1831[54], it quickly found worldwide use as an anesthetic in obstetrics, dentistry, and surgery. Military doctors have applied it to injured combatants for wound treatment since the American Civil War. Unfortunately, it is presently banned from inclusion in pharmaceuticals and cosmetic products due to its potential carcinogenicity.[53] Notwithstanding this negative label, chloroform is still used in research laboratories as well as in several carefully-monitored industrial processes.

Lipids and chloroform are chemical species with different physicochemical properties and unique historical backgrounds. The work of Professor Jordi Folch and associates in the 1940s and 1950s intertwined their histories forever.[73-80] The Folch teams spawned many lines of research with definite lipid-chloroform synergism, and these activities continue at an accelerated pace to this day.[81-91] Perhaps the biggest lesson from this story should be addressed to today's young scientists: even though they have dissimilar physical and chemical properties, lipids and chloroform have shared important common ground in the past, and can be used collaboratively and prudently to tackle challenging scientific problems in the future.

ACKNOWLEDGEMENTS

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NOMENCLATURE

Latin letters

A species A
B species B
c unsubscripted: molar density of an ideal gaseous mixture; subscripted: molar density of a pure liquid, mol/m^3
D Fickian diffusivity of a species in a binary gaseous mixture (subscripted), m^2/s
t time elapsed after starting a standard Stefan column evaporation-diffusion experiment, s
y mole fraction of a given species at a specific gas-phase location in the column (subscripted), -
z vertical coordinate with origin at the bottom of the column (see Figure 4; subscripted), m

Greek letters

λ constant defined by Equation (2), m^2/s

Subscripts

10 initial location of the liquid-gas interface
1, 2 specific column locations: 1, liquid-gas interface; 2, top
AB A-B gas pair
Az_1 species A at z_1
Az_2 species A at z_2
L liquid

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Obituary

Professor Alexander Kessenikh (1932-2021)

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On September 15, 2021, professor Alexander V. Kessenikh had passed away. He was known for his works on nuclear magnetic resonance (NMR) and history of science (Figure 1).

Alexander V. Kessenikh was born on February 13, 1932 in Tomsk, a scientific capital of Siberia at that time, where his parents-physicists worked. Due to the work on military subjects of his father, Vladimir N. Kessenikh, the family moved to Moscow Region in 1943. In 1949-1953, Alexander Kessenikh studied at the Faculty of Physics of the Lomonosov Moscow State University (MSU). It was a momentous time for both Soviet physics and Soviet student community, and Alexander Kessenikh took an active part in the events of that moment. In late 1940s-early 1950s, science in the USSR has been subjected to the strongest ideological press or even (in some scientific fields) to almost complete destruction. In physics, the relativity theory and quantum mechanics were declared "idealistic and hostile" branches of sciences. Fortunately, the burning need of the USSR for atomic weapons prevented the defeat of physics, but in MSU and many other universities of the country, modern physics education was practically destroyed. In the MSU Faculty of Physics, students rebelled with the demand to return them to a full-fledged physics education, although this was an unprecedented and threatened danger for the initiators. This resulted in a revolution in physics education at MSU (and hence in other universities) and in students' self-awareness bringing a breath of freedom to the student community. Alexander Kessenikh was one of the organizers of that revolution[1].

Figure 1. Alexander V. Kessenikh (1932-2021). Source: Personal archive of A.V. Kessenikh.

Another manifestation of freedom in the student community was the flourishing of student amateur art. In this area, Alexander Kessenikh was also one of the most popular student leaders due to his poetic talent (later, he authored several published books of poetry, e.g.[2]) that he applied not only to writing poetry: together with his like-minded friends, he wrote several humorous operas on the themes of student life and created a student festival, Archimedes Day. One of the first Archimedes Days was visited by Niels Bohr, who was delighted and said that if students were capable of the same

Figure 2. Alexander Kessenikh among the other authors of the first Physics Faculty opera and the Archimedes Day festival. Left to right: Yu. Gaponov, S. Soluyan, A.V. Kessenikh, V. Pismenny, Yu. Dnestrovsky. Around 1980. Source: Personal archive of A.V. Kessenikh.

ingenuity and wit in physics, he would feel secure about the future of physics. The Physics Faculty operas and Archimedes Days became a model for students and catalyzed the emergence of similar student festivals across the country, and not only among physicists[3,4] (Figure 2).

From his first steps in science, Alexander Kessenikh linked his scientific fate with the recently discovered NMR. His research interests included dynamically and chemically induced polarization of nuclear spins, double nuclear-nuclear resonances, paramagnetic relaxation mechanisms, and structural and chemical applications of nuclear magnetic resonance. Among his most cited works are works on the dynamic polarization of protons[5]. In this area, Alexander Kessenikh made an outstanding scientific discovery: a new (three-spin) mechanism of dynamic nuclear polarization (DNP) in solids was proposed theoretically and confirmed experimentally[5-7]. This mechanism (named "cross effect") is currently recognized as one of the main DNP methods and is successfully used in nuclear physics, NMR tomography, and other fields[8]. The priority of the Kessenikh group in this discovery is now generally recognized.

Since the late 1990s, Alexander Kessenikh started his research in the history of physics. He made a truly huge contribution to the creation of the history of research in the field of NMR in the USSR. In fact, his works constitute an encyclopedia of this history. He desired also to compose a database of literature in the field of magnetic resonance. Fortunately, he managed to publish the results of this work[9,10]. Studying the interaction of physics and chemistry in the history of NMR research allowed him to contribute to the analysis of interdisciplinarity in physics research. He also studied the social history of Soviet science focusing on the Golden Age of the Soviet science (mid-1950s-1960s) which had previously been poorly studied. The other focus was on scientific schools in physics, and his research gave a fruitful example of considering the Soviet physics history as the history of formation and development of scientific schools in physics. Along with his own scientific research in the field, he did a lot of editorial work[11-14]. Unfortunately, most of his research on history of science remained published only in Russian (e.g., in [11-14] and other collections of articles).

Despite his poor health, he did not stop working until the very last days. Thus, in recent years, he developed a fruitful cooperation with the journal Substantia, for which he wrote two articles[15,16]. Alexander Kessenikh did not live a few months to be 90.

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