March 2021
Vol. 5 - n. 1

2532-3997

Substantia

An International Journal of the History of Chemistry

FIRENZE UNIVERSITY PRESS

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An International Journal of the History of Chemistry

Vol. 5, n. 1 - 2021

Firenze University Press

***Substantia*. An International Journal of the History of Chemistry**

Published by
Firenze University Press – University of Florence, Italy
Via Cittadella, 7 - 50144 Florence - Italy
http://www.fupress.com/substantia

Direttore Responsabile: **Romeo Perrotta**, University of Florence, Italy

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Editorial

Giving Credit Where It's Due – The Complicated Practice of Scientific Authorship

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Anyone who has participated in modern scientific publishing has experienced the potentially complex issue of coauthors, both in terms of who merits to be included on a particular paper and in what order should they be listed. During the early years of serial scientific publications in the 17th and 18th centuries,[1] nearly all papers consisted of just a single author. In contrast, the growing complexity of most present-day studies has required collaborative teams to accomplish the work needed to present a suitable report meriting publication.[2] Some have attempted to tie this move to multiple-author papers with the introduction of large-scale government funding following World War II.[3,4] While valid arguments can be made about the expansion of the scientific enterprise at this point in history,[4] simply browsing the contents of prominent journals shows that papers with two or more authors predated this event and were somewhat common by the second decade of the 20th century. Of course, one can now easily find papers with 10 or more coauthors, further complicating the ability to properly recognize each author's specific contribution. As such, it is not surprising that the history of science includes various cases of authors not receiving sufficient credit for their contributions, and it was recent research into one such controversial case[5] that has led to the current discussion. Unfortunately, there exists no firm, uniform rules for determining authorship[6] and current practices can vary significantly,[7] even to the point that the literature is now plagued with ethically questionable practices such as *coercive* authorship[4,7] (senior officials requiring authorship on the work of subordinates without any contribution), *gift/honorary* authorship[4,6,8] (the addition of authors that did not actually contribute to the work out of respect or friendship), or the opposite case where actual contributors are given no credit whatsoever (sometimes referred to as *ghost* authorship[4]). As such, the goal here is to present various best practices in terms of both determining valid authorship, as well as addressing the related issue of author order on a given publication.

For most researchers, the baseline requirement for authorship is that the researcher should have provided a real contribution to the reported work. Of course, this does not mean that all contributors should be authors and the sticking point is often determining what merits authorship over a simple acknowledgement. One of the earliest attempts to define scientific authorship has been quoted as[2]

> …someone who has made significant contribution to the project through planning, conceptualization, or research design; providing, collecting, or recording data; analyzing or interpreting data; or writing and editing the manuscript.

The American Chemical Society (ACS) then introduced their own take on a definition in 1985 as a part of the society's *Ethical Guidelines to Publication of Chemical Research*. As outlined by the ACS:[9]

> The co-authors of a paper should be all those persons who have made significant scientific contributions to the work reported and who share responsibility and accountability for the results. Other contributions should be indicated in a footnote or an "Acknowledgments" section.

This definition has been retained nearly unchanged in each of the society's revised ethical guidelines since.

Of the currently available best practices, the International Committee of Medical Journal Editors (ICM-

ISSN 2532-3997 (online) | DOI: 10.36253/Substantia-1183

JE) included much of the ACS definition, while also attempting to provide more specific parameters. As such, the ICMJE recommended that to merit authorship, researchers must meet all of the following conditions:[10] *i*) Substantial contributions to the study's conception/design, data acquisition, or analysis/interpretation; *ii*) Drafting the manuscript or critical revision for intellectual content; *iii*) Approval of the final manuscript to be published; *iv*) Accountability for the accuracy/integrity of the work. Thus, per ICMJE guidelines, contributors that meet all four of these conditions should be authors, while those that meet three or less should only be given a suitable acknowledgement. Of course, while this provides a simple rubric for deciding authorship, the actual threshold for meeting points *i* and *ii* is still somewhat vague. For instance, what exactly qualifies as a substantial contribution? Such standards can vary from one discipline or research group to another.[7] Furthermore, the ICMJE guidelines reinforce the very traditional definition of an author as one who contributed to the actual writing of the manuscript and does not always allow credit for less traditional types of contributions to the published study.

An alternate, and somewhat more detailed, approach is the *Contributor Roles Taxonomy* (CRediT) introduced in 2014 and now adopted by a number of scientific publishers.[11] As outlined in Table 1, this consists of 14 various roles for potential contributions to a given publication, with each author assigned appropriate roles upon submission of the manuscript. While no guidelines are provided in terms of the extent of contribution expected of each author, this approach does provide a practical way to acknowledge the diversity of researchers' contributions to published papers, particularly in large teams, as well as the ability to clearly document how each author contributed to the work. Still, it does become easier to justify authorship over a simple acknowledgement when it is clear that a researcher has contributed via multiple different roles. Furthermore, this new taxonomy attempts to move away from the traditional author role to the broader, and more realistic, role of *contributor* (even if still commonly referred to as a paper author in practice).[11,12] Even if publishers have not explicitly adopted CRediT, many journals are now requiring that each author's contribution be explicitly described in a dedicated section of the published paper, which effectively accomplishes the same overall goal. Overall, application of at least the spirit of these best practices should help avoid the ethically questionable practices referenced above.

Table 1. Contributor Roles Taxonomy (CRediT).

Role	Description
Conceptualization	Formulation of research goals and aims
Methodology	Development or design of methodology used
Software	Programming, implementation of computer code, or testing of existing code
Validation	Verification of the replication/reproducibility of results/experiments
Formal analysis	Use of formal techniques to analyze study data
Investigation	Performance of experiments or data collection
Resources	Provision of materials, reagents, samples, etc.
Data curation	Management activities to annotate and maintain data for research and later re-use
Writing – original draft	Preparation/presentation of the published work, specifically writing the initial draft
Writing-review/editing	Critical review, commentary, or revision of the published work
Visualization	Preparation of data presentation/visualization
Supervision	Oversight and responsibility for the research
Project administration	Management and coordination of the research activity and execution
Funding acquisition	Acquisition of financial support for the project

Once decisions have been made concerning which contributors merit authorship, there is still the thorny issue of author order, particularly in publications with a significant number of authors. By far the most common practice is to list authors according to their relative contributions to the work. That is, the author with the greatest contribution is given first author status, with others ranked in descending order of contribution. The only exception to this is typically the placement of the principle investigator (PI), who is most commonly listed last and designated as the corresponding author. However, even the placement of the PI can vary and sometimes the PI can be given first author status, either as the result of providing the bulk of the contributions (as in review articles, etc.), as the result of discipline traditions or convention, or in an attempt to increase the paper's perceived exposure.

As the number of first author publications can play a critical role in job applications, extramural funding, etc.,[3,4] deciding who merits first author status can be a tricky and contentious process, particularly in cases where multiple authors have provided somewhat similar contributions to the work. As a potential solution to this issue, the practice of designating multiple first authors has started to become common, in which footnotes are used to specify that each author contributed equally to the work.[13] Even here, however, the first of the two "equal" authors still tends to receive greater recognition,

particularly where only a single author is used to refer to the work. Although less common, similar practice can also be applied to the last/corresponding author, for those studies that involve multiple PIs. In reality, however, most collaborations (at least informally) have one PI that takes the lead responsibilities on each paper, thus removing the need for multiple corresponding authors.

As a way to remove the issues of author order, some fields have adopted the practice of listing authors in alphabetical order. The problem here is that unless this is made explicitly clear to the reader, most will still assume authors are listed in terms of relative contribution, as this is the far more common practice. Another compromise is the practice of what is sometimes referred to as *negotiated order*. That is, to come to some consensus or mutual agreement between authors on the listed order. For example, in cases where two researchers have provided near equal contributions, it is likely that the people in question will both contribute to more than one publication from the same project. Therefore, the order of first and second author on one publication can then be reversed on the next publication, thus providing an avenue of giving balanced credit across the total scope of the project.

Needless to say, while this discussion has attempted to present best practices, none of these approaches are perfect and there are still plenty of opportunities of argument and contention. In the end, for everyone involved (students and advisors, alike), the best practice is to have open and transparent discussions about these issues and try to come to agreement about these decisions prior to drafting the manuscript for publication. In addition, if the author order needs to be modified due to changes in team composition or relative contributions of authors, make sure that all authors understand the reasoning behind the applied changes. In the end, open communication is the only way to limit unwanted disputes related to the authorship of a given publication.

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Substantia
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Feature Articles

History of Research on Antisense Oligonucleotide Analogs

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Abstract. In the search for novel therapeutics, *antisense oligonucleotide* (ASO) analogs have been a major focus of research for over 40 years. They use the *antisense* strategy, namely they have a nucleic acid base sequence that is complementary to a portion of a specific mRNA that is produced in the cell, or to a viral RNA, in order to selectively inhibit gene expression. Oligonucleotides need to be chemically modified to stabilize them against hydrolysis by endogenous nucleases. Until now several phosphorothioate (PS) oligonucleotide analogs have been approved by the FDA for human use. This article seeks to provide a history of this subject to date.

Keywords: Antisense, Oligonucleotide, Analogs, Therapeutic, RNA.

OPEN ACCESS

Citation: Cohen J. S. (2021) History of Research on Antisense Oligonucleotide Analogs. Substantia. *Substantia* 5(1) : 9-25. doi: 10.36253/Substantia-964

Received: Jun 09, 2020

Revised: Aug 09, 2020

Just Accepted Online: Aug 11, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

INTRODUCTION

The basic premise of the antisense approach to therapy is that since most human diseases are genetic in origin, it is necessary to use a genetic means to cure them. This includes cancer and infectious diseases, being the insertion of bacterial or viral genes into cells. Antisense depends on the intervention of information-containing drugs in the form of synthetic oligonucleotides to disrupt the flow of information transfer from DNA to RNA to protein.[1] Since DNA is highly protected, the process of transcription from DNA to mRNA is more difficult to target,[2] so the process of protein biosynthesis is the easier to target, hence antisense is a form of *translation arrest* in which the mRNA is targeted (Figure 1). Whereas usual drugs bind strongly to a protein active site and inhibit its action, the antisense approach is intended to prevent expression of the same protein by blocking its synthesis at the basic molecular level using Watson-Crick base pairing.

Antisense is a term that was introduced following the description of the double helical structure of DNA by Watson and Crick,[3] the *sense* strand being the one that is expressed into protein and the *antisense* strand being its unexpressed complement. The *first* published use of an oligonucleotide as an antisense inhibitor was by Paul Zamecnik and Mary L. Stephenson against Rous sarcoma virus in 1978.[4,5] This was before the advent of DNA synthesizers, so they had a natural phosphodiester (PO) 13-mer oligo[6] synthesized

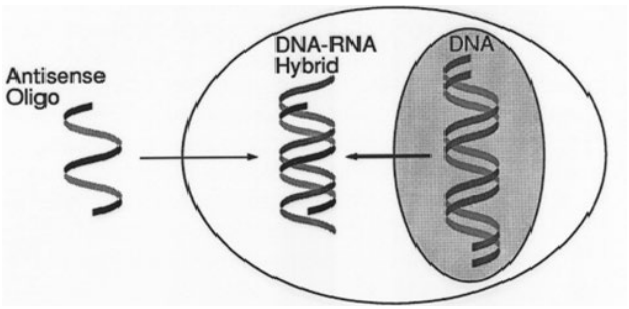
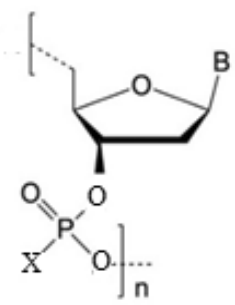


Figure 1. Schematic showing translation arrest by an antisense oligo binding to a cellular mRNA.



Name of Analogue	X
DNA	O
Phosphorothioate	S
Methylphosphonate	CH_3
Phosphoramidate	NH_2

Figure 2. DNA analogs based on minor substituents of the canonical structure.

manually for them that was complementary to a 3' and 5' reiterated terminal region, and they found it inhibited virus production. The use of a natural PO oligo no doubt resulted in hydrolysis to some extent by nucleases in the system.[7] Other work was reported on this antisense approach using specific sequence PO oligos.[8,9] Zamecnik was awarded the National Medal of Science in 1991 for his work on antisense among other things.

This story began for me in 1986,[10] when a paper appeared in *Biochemistry* reporting the use of a chemically modified antisense oligonucleotide (ASO) analog inhibiting stomatitis virus infection from Paul Ts'o and Paul Miller at Johns Hopkins University.[11] They used a methylphosphonate (PM) analog (Figure 2) to stabilize the oligo against endogenous nucleases in the cell. I was aware of this work from a symposium we had both attended in Jerusalem the previous year[12] and from their previous published work.[13] Note however that their use of 9-mers meant that they were unlikely to have bound very effectively as a duplex with the target RNA.

The potential of this approach against HIV and cancer was clear, and so because of my background and experience in DNA chemistry,[14] I was assigned to develop this approach in the NCI by my Lab and Division Chiefs.[15] I began a collaboration with my colleague Gerald Zon, Bureau of Biologics, FDA, since he was b-testing a prototype Applied Biosystems automated DNA synthesizer in his lab.[16,17]

Serendipitously, Zon and his colleagues were synthesizing phosphorothioate (PS) analogs (Figure 2) of oligos to identify signals in the ^{31}P NMR spectrum.[18] Phosphorothioate poly-ribonucleotides had been described by Eckstein and co-workers,[19] and their resistance to hydrolysis by nucleases had been noted,[20,21] but no-one before had synthesized specific sequence PS oligomers for a therapeutic application. We tested several PS oligos targeted against the *rev* gene mRNA of HIV in collaboration with Sam Broder, Head of the Oncology Program at NCI, using the PM and PS analogs with the natural phosphate (PO) oligos as controls. The *rev* gene (also known as *art/trs*) was chosen because it produces the *gag* protein that can easily be quantified. We used 28-mers since according to the calculations of Hélène and Toulmé this should reduce the probability to a single target sequence in the human genome and would be long enough to duplex.[22] This had consequences since at that length the PM oligos, which are uncharged and hydrophobic, were virtually insoluble and gave very low yields.

By contrast, the PO and PS analogs are charged and are water soluble. However, at first the yield of PS analog was low due to the inefficiency of the sulfurization reaction[23] compared to the usual oxidation reaction.[24,25] We were able to increase the yield using a different solvent.[26] The oligomers were tested in an HIV assay and the PS oligomers showed excellent inhibition against the *rev* gene, but *none* with the PM oligos or the PO oligo controls.[27] However, disturbingly we also found inhibition by the control sequence PS compounds, as well as PS homo-oligomers. This led to further research using a more appropriate assay system using chronically infected cells that gave a pure sequence-dependent antisense inhibition, with the same controls as before (Figure 3).[28]

Zon and his collaborators carried out a comparative study of inhibition of the chloramphenicol acetyltransferase (CAT) gene in a plasmid by various oligo analogs (phosphodiester, methylphosphonate, alkyltriester and phosphorothioate) and found that the PS analog was the most effective.[29] Zamecnik and his colleagues also applied PS and phosphoramidate oligo analogs to HIV.[30] We also compared the inhibition by PS oligos against the *gag, pol* and *rev* genes of HIV[31] and we also extended the use of PS oligos against other viruses.[32] We also expanded our work with PS oligos to the selective inhibition of oncogenes, c-*myc* in hematopoietic cells in culture by liposome fusion[33] and *bcl*-2 in leukemic cells.[34]

After these initial results were reported there was an immediate reaction.[35] Several companies were established[36] (**Table 1**), with billions of dollars of investment,

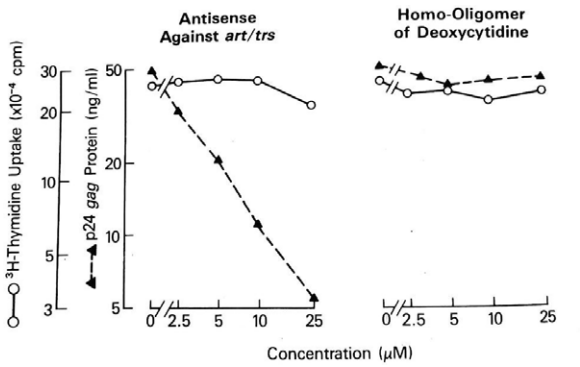


Figure 3. Sequence-specific inhibition of HIV p24 *gag* protein expression after 5 days in culture supernatant by the antisense phosphorothioate 28 mer against *rev* (5'-dTCG TCG CGT TCT CCG CTT CCT GCC A) determined using a radioimmunoassay (left). Neither the normal oligo, the sense phosphorothioate nor the homo-oligomer S-dC_{28} (right) had any inhibitory effect.[28]

to exploit the finding of stable PS oligos as therapeutic agents, which was patented by NIH.[37] Several of the original companies were subsequently taken over by larger companies and also diversified their products to other than antisense compounds.

I organized one of first conferences on this subject entitled "Oligodeoxynucleotides as Antisense Inhibitors of Gene Expression: Therapeutic Implications," that was sponsored by NCI and NIAID and held in Rockville, MD, on June 18-21, 1989.[38] About 300 people attended, including most of those then active in the field. Many companies supported the conference and the dinner speaker was Michael Riordan of Gilead Sciences, whose subject was "Oligos: a commercial proposition." Other early conferences on this topic were held in the UK (Cambridge, organized by Dan Brown, 1987), France (Les Arc, organized by Jean-Jacques Toulmé, 1988), Russia (Akademgorodok, organized by Valentin Vlassov, 1988).[39]

If one searches the scientific literature (using CAS SciFinder) for "antisense AND oligonucleotide" there are some 43,000 hits, with a gradual increase from 1987 (15 hits) to 2001 (3,249) and then a gradual decline to 2019 (1,086) (Figure 4). Explaining this histogram is one aspect of the function of this article. Clearly it would be impossible to do justice to all these many articles, reviews (4,837) and other publications on this topic. However, it is the responsibility of the author to try to discern trends in this morass of data.

In 1989 we published the first volume on this subject, entitled "Oligodeoxynucleotides: Antisense Inhibitors of Gene Expression,"[41] which contained chapters from all the leading researchers in the field. This is an

Table 1. Companies engaged in antisense and DNA therapeutic R&D.*

Company	Location	Comment
Akcea Therapeutics	Boston MA	Owned by Ionis
Biogen	Cambridge MA	
Codex	San Diego CA	
Dynacure	Illkirch, France	
Dynavax Technologies	Emeryville CA	
Genta Inc.	San Diego CA	
Gilead Sciences	Foster City CA	Founded 1987
Helix Naonotechnologies	Cambridge MA	
Hybridon	Worcester MA	Acquired by Idera in 2004
Ionis Pharmaceuticals	Carlsbad CA	Originally ISIS Pharma
NapaJen Pharma	Burlingame CA	Japanese company
Pharmagenics	Allendale NJ	Acquired by Genzyme in 1997
Procarta Biosystems	Norwich UK	
Ranger Biotechnologies	Funen Denmark	
RogCon Biosciences	San Diego CA	
Secarna Pharmaceuticals	Martinsreid Germany	
Sterna Biologicals	Marburg Germany	
Stoke Therapeutics	Bedford MA	
Triplex Pharmaceuticals	Woodlands TX	Acquired by Argus in 1995
Zata Pharmaceuticals	Worcester MA	

* This is not a complete list.

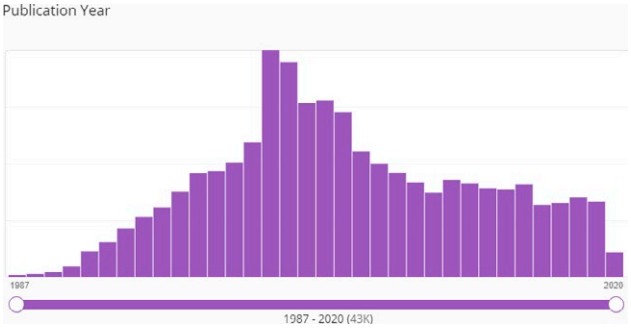


Figure 4. Number of publications per year for the literature search "antisense AND oligonucleotides." The discovery of the PS analog of oligos as a stable (slowly hydrolysable) and water-soluble (hydrophilic) alternative to the natural PO compounds, made the antisense oligo approach feasible for human therapy,[40] and led to the initial *increase* in published work on this subject.

Table 2. First Generation Oligonucleotide Analogs.

Analog	Designation	Structure	Reference
Phosphate	PO	(RO)(R'O)(HO)-P=O	Zamecnik et al., 1978[4]
Methylphosphonate	PM	(RO)(R'O)(CH3)-P=O	Agris et al., 1986[11] Tidd et al. 1988[47]
Phosphorothioate	PS	(RO)(R'O)(HO)-P=S	Matsukura et al,, 1987[27]
a-anomer	a-PO		Morvan et al., 1987[48] Rayner et al., 1990 [49]
Phosphoroselenoate	PSe	(RO)(R'O)(HO)-P=Se	Mori et al., 1989[50]
Phosphotriesters	---	(RO)(R'O)(R"O)-P=O	Miller et al., 1974[51]
Phosphorodithioate	PS2	(RO)(R'O)(HS)-P=S	Jaroszewski et al., 1996[52]
Phosphoramidate	PNH2	(RO)(R'O)-P(O)-NH2	Agrawal et al., 1988[30] Peyrotte et al. 1996[53]

excellent summary of the state of affairs in this subject at that time. A series of alternative oligo analogs were described that have come to be called "first generation analogs." (Table 2) and note that they were mostly developed prior to 1990.

Mixed alternating co-polymers, such as (PO-PS) n[42] and (PM-PS)n,[43] and end-protected analogs on natural PO oligos to protect against exonucleases[44] were also described. Since the PS analog appeared at that time to be the best analog available for truly therapeutic purposes, many applications were made with them, including clinical trials and applications to the FDA for human use.[45,46]

PROBLEMS WITH OLIGONUCLEOTIDE ANALOGS

Several factors can mainly be blamed for the subsequent gradual *decrease* in published work on antisense oligo analogs after 2001 (**Figure 4**): first, problems that arose in the application of the PS and other ASO analogs as therapeutic agents, including: 1. cellular uptake; 2. non-sequence dependent effects; 3. RNase H function; 4. cost of production; 5. primary, secondary and tertiary structure effects of oligos. The second cause of the reduction in interest in synthetic oligo analogs was the finding of endogenous antisense mechanisms within living cells, mainly so-called silencing RNA (siRNA). We will consider each of these factors as a part of the history of the development and application of ASO analogs.

1. Cellular Uptake:

Since the antisense mechanism occurs within the cell cytoplasm, it is essential that the putative inhibitory oligo must be able to enter the cell. This subject was considered early on in the history of antisense oligos.[54] There are three means for cellular uptake of oligos: a. passive diffusion through the cell membrane; b. active transport via a specific membrane mechanism; and c. specific means of delivery, for example either encapsulating the oligo within liposomes, or attaching a membrane–active agent.

a. *Passive diffusion*: This usually only occurs for small hydrophobic compounds that dissolve in the membrane lipid bilayer, and it is unlikely for any oligomer, least of all a negatively charged one to be able to enter the cell by passive diffusion.

b. *Active transport*: A fluorescent label acridine was attached to the 5' end of oligos, in this case PS oligos in order to avoid cellular degradation, and the uptake into the cell was monitored over time. After 24 hours a punctate distribution (i.e discrete points of fluorescence) was observed, and this was taken to mean that uptake not only occurred, but that it was by the formation of vesicles within the cells (non-vesicular up-take would give a homogeneous distribution) (Figure 5).[54]

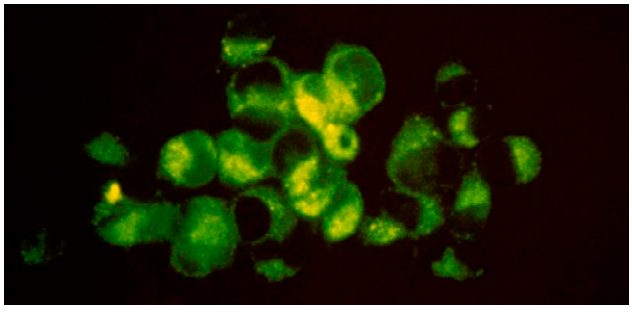


Figure 5. Photomicrograph showing punctate distribution of fluorescently labelled PS oligo within cells after 24 hrs exposure.[54]

c. *Delivery mechanisms*: The delivery of ASO into cells using liposomes was studied in detail by Yehezkel Barenholtz and co-workers, who investigated issues of sequence dependence, charge, size and composition of the liposomes, and studied comparative inhibition of the bcl-2 oncogene using different compositions of liposomes.[55-57] The authors concluded that liposomes are an effective means of improved delivery of ASOs into cells. Other delivery mechanisms include linking the ASO with membrane-active peptides to enhance cellular uptake of ASOs,[58] and binding to gold nanoparticles.[59] This general subject has been reviewed.[60,61]

2. Non-Sequence Dependent Effects

Non-sequence dependent inhibition was observed with phosphorothioate oligomers in the first study carried out with HIV.[27] In fact homo-oligomers of cytosine such as S-dC_{18} were very efficient inhibitors of HIV replication. The mechanism of this inhibition was investigated in detail, which showed that the intact poly-anion was the active agent.[62] These homo-polymers were also applied as inhibitors of other viruses.[63,64] Although these non-sequence dependent effects in viruses were at first considered a potential problem for the application of PS-oligos to cellular systems for the inhibition of oncogenes, this effect was never reported as a problem.

Another known problem is the antigenic effect of the sequence CpG in oligos, but this has been studied and can be avoided with substituents on the C base.[65-67]

3. RNase H Function

RNase H is an enzyme that specifically cleaves the RNA molecule in a DNA-RNA duplex.[68,69] Therefore, it should enhance the effectiveness of an ASO relative to purely passive binding and inhibition. However, not all DNA analogs are substrates for RNase H.[70] The PS oligo analog is a substrate,[71] but it depends on the number of PS groups present, if there are too many then the oligo can become inhibitory for RNase H action.[72] On the other hand, Summerton has argued that having an RNase H-independent antisense function of morpholino ASOs is an advantage.[73]

4. Cost of production

It was understood from the start that ASOs are large molecules compared to usual drugs, and to produce them would cost much more. At first the cost per nucleotide for automated synthesis was prohibitive, but over time, savings in chemicals and methodology greatly reduced the cost,[74] so that now it is certainly affordable.

5. Primary, secondary and tertiary effects of oligos

Oligonucleotides are not always simply in a denatured conformation, but can form secondary and tertiary structures, such as hairpin loops and tetra-G complexes. How these can affect the inhibitory function of the intended ASO is not always easy to determine, but generally hairpin-loops have been proposed to be an aid to the protection of vulnerable oligos (mainly PO) against nucleases.[75,76]

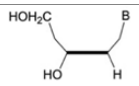
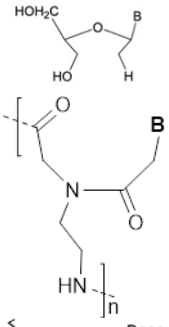
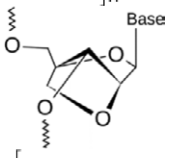
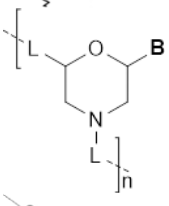
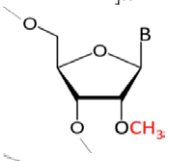
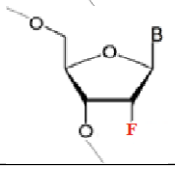
6. siRNA and its implications for ASO applications

In 2006, the Nobel Prize in Physiology or Medicine was awarded jointly to Andrew Fire and Craig Mello for their discovery of RNA interference – gene silencing by RNA.[77] It was realized that the large number of small RNA molecules, micro-RNAs, in the cell perform in effect an endogenous antisense function.[78] This finding resulted in a competitive approach to the use of antisense oligonucleotides, namely the use of the so-called silencing or small interfering RNA, known as siRNA.[79] It was soon realized that siRNA could be exploited either biologically or chemically,[80] in the form of nuclease-protected ribo-oligonucleotide analogs, much like ASOs. Currently there are drugs based on RNAi being developed.[81] It was also reported that there are synergistic effects between ASO analogs and siRNA inhibitors.[82] There are problems with control of the siRNA approach,[83] and the ASO methodology seems more "druggable." However, further consideration of this topic is beyond the scope of this article.

SECOND GENERATION OLIGONUCLEOTIDE ANALOGS

Because of the problems experienced with the PS analog, that was the first effective ASO developed, and because of the general desire to find more effective drug candidates, "second- generation analogs" were also subsequently developed, mostly after 1990 (Table 3). Here we will describe the most important of these and assess their potential as therapeutic agents relative to the first-generation ones, particularly the PS analog.

Table 3. Second Generation Oligonucleotide Analogs.

Name	Designation	Structure	References
Acyclic derivatives	___		Vandendriessche et al., 1993[84]
Peptide NA	PNA		Egholm et al., 1992[85]
Locked NA	LNA		Wengel et al., 1999[86]
Morpholino NA	___		Summerton & Weller, 1997[87]
2'O-Me RNA	___		Wagner et al., 1991[88]
2'-Fluoro RNA	___		Eckstein et al., 1991[89]

1. Acyclic nucleic acids

There have been many attempts to prepare oligomers containing ring-opened furanoside, as shown in Table 3 (opened between C1' and C4' or C2' and C3').[90] Piet Herdewijn and co-workers have examined a series of these compounds[91] and have concluded that they are too flexible to allow the formation of stable duplexes with a target DNA or RNA.[84] GNA is the oligomer in which glycol has been used to replace the ribo-sugar,[92] however this was found not to duplex with RNA, although it did form a strong homo-duplex[93] (Figure 6).

2. Covalently linked oligos

By attaching a specific chemical group to (usually) the 5'-terminus of an oligonucleotide intended for antisense inhibition, researchers have hoped to either increase its efficacy, improve its ability to enter cells, or actually carry out a catalytic reaction. The first known use of a "complementary addressed" oligo to chemically modify a tRNA was from the lab of Dr. D.G. Knorre and V.V. Vlassov.[94]

An acridine ring attached to either end of an oligo can be a reporter group of the environment, both in the isolated oligo (that might form secondary structure) or in the formation of a duplex, where is might interca-

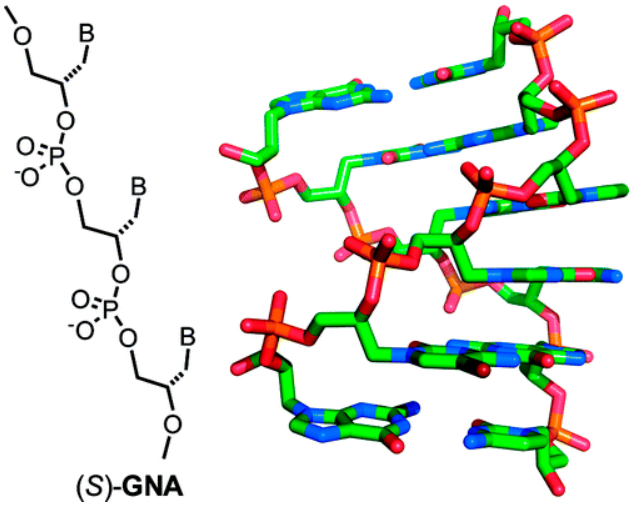


Figure 6. Homo-duplex formed by glycol nucleic acid (GNA).[93]

late between the base pairs.[95] Fluorescent acridine covalently linked to an oligo was also used to follow cellular uptake.[96] As well as several passive aromatic groups that can be attached, it is possible to attach reactive groups meant to cleave the RNA in a duplex, groups such as EDTA-Fe(II)[97-99] or porphyrin with iron.[100] Knorre et al. provide an extensive list of such reactive groups.[101] We attached imidazole to the terminus of an oligonucleotide[102] in order to try to reproduce the catalytic effect of RNase, since the catalytic effect depends on imidazoles in the active site.[103] Since then much progress has been made in synthesizing oligos containing one,[104-106] two[107] or multiple imidazole groups[108] to mimic the catalytic activity of ribonuclease using tRNA as a target. This approach has great potential for site-specific RNA cleavage.

3. Peptide nucleic acids

Oligopeptides with nucleic acid bases in place of the usual amino acid side chains have been developed for antisense applications[85] (Table 3). Their advantage is that they are of course resistant to nucleases and are water soluble, and they can easily be attached to cell-penetrating peptides in order to improve cell uptake.[109,110] A variety of biological systems, including bacteria have been targeted successfully with antisense PNAs.[111]

4. Locked nucleic acids

Locked Nucleic Acids (LNA) were developed in order to provide an analog completely resistant to nucleases, and by virtue of the locked structure of the sugar moiety (Table 3), reduce the flexibility of the ribo-furanoside ring, thus improving duplex stability.[86] Kurreck et al compared the LNAs with the PS oligos and found them to be superior, although this does not take into account the cost and difficulty of production.[112] Another report declared the LNAs were superior to other ASOs.[113]

5. Morpholino nucleic acids

Summerton and his co-workers developed the morpholino analog of oligos as a mimic of the natural structure (Table 3), and claim that they are superior to other analogs.[87] In studies of antisense activity and RNase H function they claim that the morpholino analog exceeds the function of the PS analog.[73,114] In a comparison of the PNA, LNA, and morpholino, it was found that each had advantages.[115] The morpholino analogs have been applied therapeutically in many different biological systems.[116]

6. Modified RNAs – 2'O substituted analogs

Researchers realized early on that a potential source of ASOs was the ribo-oligomer with the 2'-hydroxy position substituted with a methyl group or another alkyl group, and this has become a major area of research and development. Sproat and co-workers described the synthesis of 2'O-R analogs, where R=Me, allyl or dimethylallyl, for antisense applications, primarily because these compounds were relatively easy to synthesize and are resistant to nucleases.[117] Ohtsuka and co-workers used 2'-O-Me analogs to inhibit the expression of portions of the human β-globin gene.[118]

Lamond and Sprout expanded on the use of the 2'O-alky-lRNA analogs for applications in RNA biochemistry.[119]

Cook and co-workers described the use of combined 2'O-alkyl ribo- and deoxy- co-polymers and showed that they increased RNase H activity.[120] And others combined the PS oligos with 2'O-alkyl co-polymers as improved chimeric antisense analogs.[121,122] In a comparative study for muscular dystrophy drugs, the 2'OMe-PS combination was found to be more effective in inhibition of exon skipping than other ASOs including LNA and PNA.[123] This approach of "chimeric" combinations of analogs for improved RNase H degradation of the mRNA complex was also described by Giles and Tidd.[124] Eckstein and co-workers described the presence of a 2'-fluoro adenosine in a ribozyme[89] and Cook and co-workers synthesized uniformly modified 2'-deoxy-2'-fluoro PS oligos as nucle-

ase-resistant antisense compounds with high affinity and specificity for RNA targets.[125]

This approach was expanded upon by Stan Crooke and those at Ionis (formerly ISIS), who favor 2'O-methoxyethyl substituents (MOE) and 2'4'-constrained ethyl (cET) like the LNA and GalNAC conjugates of ASOs.[126] Their collaborators at Ciba-Geigy formulated the concept of gapmers as second generation OAS analogs, with the "wings" consisting of 2'O-alkyl PS analogs and the center "gap" consisting of PS oligo only, in order to take advantage of the increased RNase H activity.[127] Of course there are a myriad variations on these themes, but their general conclusion is that the future is with RNA oligo analogs. It should be pointed out, that many of the applications of Crooke and co-workers are targeted not at mRNA *per se*, but at pre-mRNA before splicing, and at introns or exon-intron junctions.[128] This provides unique target sequences that prove to be extremely efficient for correcting abnormal gene expression.

OTHER RELATED APPROACHES

1. Triple Helix

The formation of a triple stranded helix with a strand of an oligonucleotide binding into the major groove of the DNA duplex by Hoogsteen base pairing was first shown in 1968 [129] (Figure 7).

Dervan and coworkers showed in 1987 that an oligonucleotide with an EDTA-(Fe(II) attached could bind in the major groove of DNA and cause a double strand break.[130,131] Other workers also added to this approach as a so-called *anti-gene strategy*.[132] Hogan and co-workers sought to make triple helix formation an effective gene targeting technique.[133] Although triple helix formation has continued as an active area of research its therapeutic promise has not been realized.

2. Aptamers

Aptamers are oligonucleotides (or peptides) that bind to a specific target molecule and are selected by repetition from a large random sequence pool that was first described in 1990.[134-136] Many applications of this technique were made to evolve oligonucleotides that bind to various biological molecules.[137-140] Of more recent interesting applications are improvements in the methodology and approaches to therapeutic applications.[141-143]

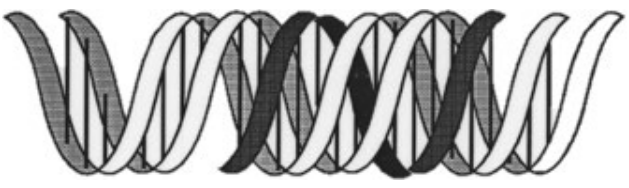


Figure 7. Triple helix showing an oligo (dark) bound in the major groove of DNA.

3. Ribozymes

Ribozymes are catalytic RNA molecules that were first described in the early 1980's as the active enzymatic component of RNase P.[144-146] There are various types of ribozymes that have been characterized from their general shape as hammerhead, pistol, twister and hairpin ribozymes.[147] Therapeutic applications of ribozymes have been described.[148-150]

4. Effects of mRNA structure

In considering the antisense mechanism, one must always bear in mind not only the antisense oligonucleotide analog, but also the mRNA being targeted. It is not enough to know the sequence of the mRNA/DNA, but also its conformation to ensure that the oligo will be able to access a target sense sequence. Studies have been made on the conformation of mRNAs and the access to target sequences.[151,152] Vlassov and co-workers showed that a binding oligonucleotide can modify and invade the structure of a tRNA target, thus making their use potentially wider.[153] It has been proposed that a statistical analysis can improve the ability to choose target sequences in mRNA.[154]

5. Molecular Dynamics Simulations

Previously we used molecular dynamics (MD) and energy minimization protocols to assess the duplexation of a PS oligo with target complementary RNA *in silico*.[155] MD calculations have also been applied to the PNA analog.[156] Recently our earlier methodology has been refined and updated [157] to assess the ability of previously unknown oligo analogs to dimerize with a target RNA. It is not generally realized that there are 10 atoms between one base and the next in DNA (Figure 8), all of which are not required for dimerization to occur in principle.

Of more radically modified analogs that can be conceived with methylene groups in place of the sugar moiety, only one has previously been characterized, namely

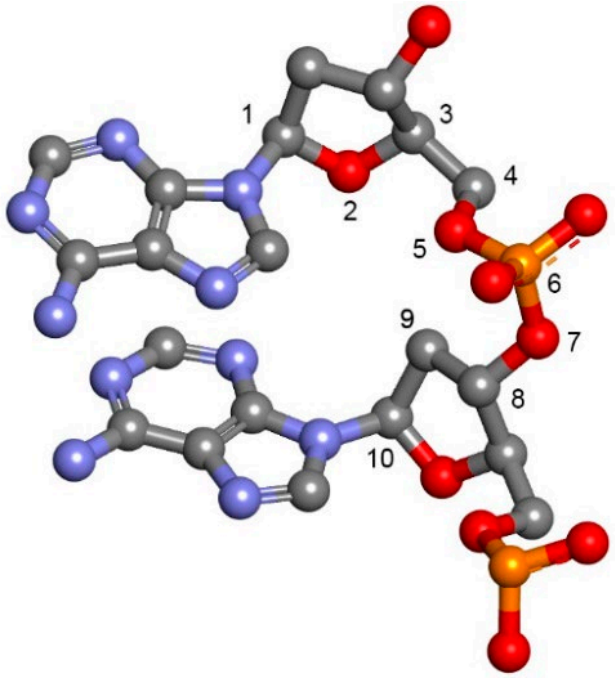


Figure 8. Showing the number of atoms/bonds between adjacent bases in DNA.

GNA.[93] Recently we have used this MD methodology to assess the capability of other analogs to duplex with complementary DNA and RNA, and we have found that only one of the eight possible structures does in fact form a viable duplex, namely that with the formulation [–CH(B)-O-P(O)(OH)-O-CH(B)-]$_n$ with RNA (Figure 9).[158] This use of computer aided molecular design (CAMD) to guide synthetic strategies is a viable predictive tool in future comparative ASO analog development and rational drug design.

CLINICAL ASPECTS

Agrawal and coworkers carried out a series of studies on *in vivo* distribution of various ASOs in animal models.[44,159-161] In 1998, the first antisense oligonucleotide, Fomivirsen, (Vitravene), a 21-mer PS oligo analog produced by Isis Pharma (now Ionis), was approved by the FDA for human ocular use against cytomegalovirus retinitis in immunocompromised AIDS patients. A detailed review of the recent FDA files[162] shows that there have been 6 ASOs approved for human use, three of them PS analogs, one a chimeric 2'OMe-PS combination and the two others were of other compositions. Among these drugs approved by the FDA are Nusinersen and Eteplirsen that are exon-skipping antisense oligonucleotides for the treatment of Spinal Muscular Atrophy and Duchenne Muscular Dystrophy.[163,164]

MicroRNA (abbreviated miRNA) are small noncoding RNA molecules (containing about 22 nucleotides) found in animals, plants, and some viruses, that

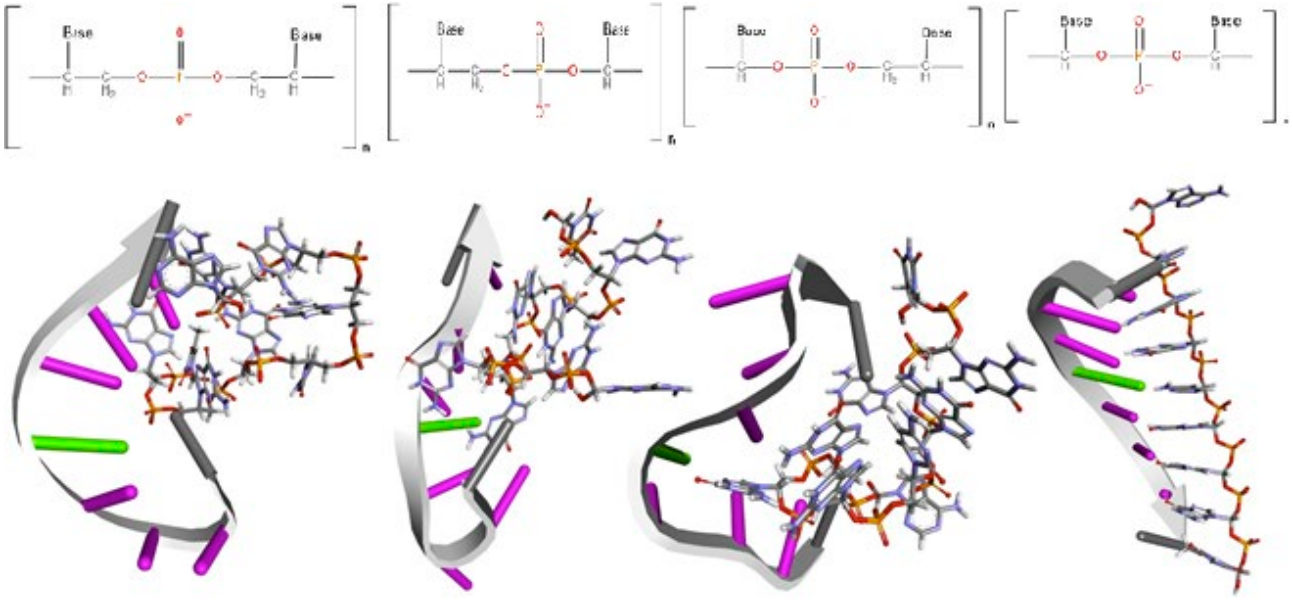


Figure 9. Representative structures from Molecular Dynamics analysis of the most populated cluster for each analog represented above, with complementary RNA. Analysis performed using the entire sampled data. Only one of the four structures with methylene groups in place of the ribo-furanoside forms a stable duplex (on the right).[158]

function in RNA silencing and post-transcriptional regulation of gene expression. miRNAs function via base-pairing with complementary sequences within mRNA molecules. miRNAs are similar to siRNAs but have different origins and specific functions. miRNAs are associated with many human diseases. miRNAs have been inhibited using locked, morpholino or 2'OMe oligos.[165,166] This is only the beginning of such inhibitions of miRNAs related to human disease.

In 1998, there were 8 PS-ASOs undergoing clinical trials.[46] A search of the NIH web-site ClinicalTrials.gov reveals the following interesting results: (a) there are 52 active or completed studies of siRNA; (b) there are 77 active or completed studies of "antisense oligonucleotides" (type not specified; also listed as AS ODNs), the majority in the US, Canada and Europe. It is to be hoped that many more will follow. It is considered possible that mixtures of antisense oligos could be used to target genetically heterogenous diseases such as cancer.

CONCLUSION

The general conclusion of this article is that the history of this subject really began dramatically with the first publication using the phosphorothioate (PS) analog of oligonucleotides against HIV in 1987.[27] For the first time this made their development as therapeutic agents feasible. This area of research then expanded rapidly, reached a peak around 2001, during which time many other analogs were developed and applied. Research then declined for two main reasons, first problems that were encountered in the application of the ASO's, such as cell uptake and cost, and secondly the development of endogenous RNAi methods that were considered to be superior. However, like other forms of endogenous biological therapy, such as gene therapy, the promise of siRNA and other techniques have also lost their initial promise. Therapeutic applications of ASO analogs is still a subject with a compelling future.

ACKNOWLEDGEMENTS

I wish to thank Frank Portugal for reading and commenting on this ms and Gerald Zon for very helpful suggestions. I am grateful to those who worked with me over a period of 34 years on this subject in NCI/NIH, Georgetown Medical School, Sheba Medical Center, Hebrew University and now at Ben Gurion University. I would also like to acknowledge the many colleagues who made significant contributions to this field, namely the late Paul Zamecnik, Samuel Broder, Paul Ts'o, Paul Miller, John Goodchild, the late Dan Brown, Eric Wickstrom, Sudhir Agrawal, Fritz Eckstein, John J. Rossi, Michael Riordan, the late Claude Hélène, Jean-Jacques Toulmé, Michael Hogan, Valentin Vlassov, Jean-Louis Imbach, Piet Herdewijn, Peter Dervan and many others. Herein I have tried to set down the record of the development of this subject as accurately as I could, and any inaccuracy is inadvertent. Note that in writing this article I have generally tried to avoid reference to patents and review articles. I thank Dr. Barak Akabayov for hosting me at Ben Gurion University.

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16. I had met Gerry Zon thru my post-doc Bill Egan, and when I was on sabbatical at the Weizmann Inst., Rehovot, Israel, in 1976-7, I agreed for him to use my laboratory at NIH for their joint research. Later in 1986 we shared a post-doc Kazuo Shinozuka.
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Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Feature Articles

Chemistry, Cyclophosphamide, Cancer Chemotherapy, and Serendipity: Sixty Years On

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OPEN ACCESS

Citation: Zon G. (2021) Chemistry, Cyclophosphamide, Cancer Chemotherapy, and Serendipity: Sixty Years On. *Substantia* 5(1) : 27-42. doi: 10.36253/Substantia-1115

Received: Oct 13, 2020

Revised: Dec 02, 2020

Just Accepted Online: Dec 04, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Abstract. *Cambridge Dictionary*: **serendipity** | *noun* | the phenomenon of finding interesting or valuable things by chance.
The year 2019 marked the 60th anniversary of the approval of cyclophosphamide (CP) as an anticancer drug by the U.S. Food & Drug Administration in 1959 for the treatment of lymphoma. Between 1959 and 2019 there were ~50,000 publications listed in PubMed that have CP in the title and/or abstract, with these annual numbers showing a continual increase, and over 1,800 such articles in 2019 alone. The discovery of CP is a prime example of serendipity in science, which also applies to key elements of the metabolism and pharmacological basis for the specificity of the cytotoxicity of CP toward cancer cells. Phosphoramide mustard (PM), $HO(H_2N)P(O)N(CH_2CH_2Cl)_2$, the principal metabolite of CP with DNA alkylating activity, was synthesized and reported by Friedman and Seligman in 1954 prior to the discovery of CP. Interestingly, the original drug design premise for synthesizing PM, which was based on elevated phosphamidase enzyme activity in cancer cells proved to be incorrect. While this wrong premise also led to the synthesis of CP, as a six-membered ring cyclic phosphamidase-activated precursor of PM, the actual metabolic conversion of CP to PM was subsequently found to involve a surprisingly complex array of metabolites and metabolic pathways, all completely unrelated to phosphamidase. Although the molecular structure of CP has an asymmetrically substituted, i.e. chiral phosphorus center, the racemic mixture of the *R*p and *S*p enantiomers of CP was used throughout its initial investigations and subsequent clinical trials despite the involvement of an initial enzyme-mediated metabolic activation step, which could, in principle, be stereoselective for only one of the enantiomers of CP. Stereochemical investigations along those lines were eventually carried out, but the results did not warrant replacement of racemic CP with either enantiomer in the clinic. Amazingly, there are now ~4,000 structural congeners of PM listed in *Chemical Abstracts*, but none have led to an anticancer drug superior to CP. This account provides a synopsis of the key chemistry and stereochemistry investigations that comprise this story of CP, as a remarkable instance of serendipity in science, and my chance involvement in the unfolding of this fascinating story.

Keywords: cyclophosphamide, cancer, metabolism, synthesis, stereochemistry.

INTRODUCTION

As part of U.S. President Richard M. Nixon's pledge in 1971 to launch an intensive campaign to find a cure for cancer, the National Cancer Act,

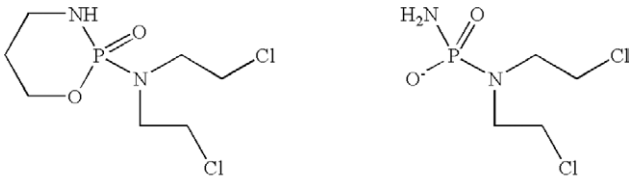


Figure 1. Structures of cyclophosphamide (CP), not showing absolute stereochemical configurations of its *R*p and *S*p enantiomers, and the deprotonated conjugate base form of phosphoramide mustard (PM).

popularly referred to as "The War on Cancer," gave the National Cancer Institute (NCI) unique autonomy and, importantly, budgetary discretion to increase its efforts to acquire new compounds for testing.[1] Knowing this and, as a newly hired Assistant Professor of organic chemistry at the Catholic University of America (CUA) in need of obtaining my first research grant, I began extensive reading about then existent anticancer drugs. Late one evening in the CUA chemistry department library, I came across a listing of cyclophosphamide (CP) together with its structure (Figure 1), the chirality of which at phosphorus immediately caught my eye, having recently trained in mechanistic organophosphorus stereochemistry as a predoctoral student with Prof. Kurt Mislow at Princeton University just several years earlier.[2]

Being chiral, the instantaneous question in my mind was whether CP was administered to cancer patients in enantiomerically pure form [i.e. optically pure (+)- or (-)-CP] or as a racemic mixture of both enantiomers [i.e. (±)-CP]. The later was the case, which was intriguing to me in view of the then known need for enzyme-mediated metabolic "activation" of CP *in vivo*, and the fact that many different types of enzymes were known to be stereoselective for one enantiomer over the other. My second question was sparked by the structural simplicity of CP compared to most of the other U.S. FDA-approved cancer drugs at the time, namely, methotrexate (1959), vincristine (1963), actinomycin (1964), and vinblastine (1965). What was so special about the structure of CP based on the then available literature?

Investigating these basic questions about CP led to my subsequent participation in The War on Cancer as an active "chemist combatant," so to speak, during the next ~15 years via several NCI-funded research grants focused on the synthesis of CP enantiomers for mechanistic and kinetic studies of CP and its metabolites using Fourier-transform nuclear magnetic resonance (NMR), which was then a relatively new and powerful analytical tool. Fortunately, this was especially true for organophosphorus compounds, such as CP, since ^{31}P, an NMR-active isotope of phosphorus, comprises 100% of the natural abundance of phosphorus. Before providing key findings from that work, and findings by others, the next section provides a brief synopsis of some of the history of CP prior to the 1970s. Another first-hand account of the earlier history of CP with a different perspective was published on CP's 30th anniversary in 1989 by Norbert Brock,[3] who discovered CP, as discussed in the next section.

EARLY HISTORY OF CP

The history of CP can be traced back to the December 2, 1943, bombing of Allied ships in the harbor of the Italian town of Bari (Figure 2) that led to a massive explosion of the SS *John Harvey*, a U.S. World War II Liberty ship, which released 130,000 pounds of "mustard gas," $S(CH_2CH_2Cl)_2$. A total of 628 military victims were hospitalized with mustard gas symptoms, and by the end of the month, 83 of them had died. The number of civilian casualties, thought to have been even greater, could not be accurately gauged since most had evacuated the city to seek shelter with relatives. This horrific tragedy led to lengthy investigations as to the source of this warfare agent and its biological effects by Lt. Col. Stewart Alexander, a medical officer attached to the staff of Gen. Dwight D. Eisenhower, and by Col. Cornelius P. Rhoads, chief of the Medical Division of the Chemical Warfare Service.[4-5] The secret shipment had most likely been destined for a chemical stockpile at Foggia, 75 miles away, in order to provide the Allied forces with the capability to retaliate against a German chemical attack.[4] Armed with the Bari report by Alexander, and the results of a top-secret Yale University study that demonstrated for the first time that a careful regimen of intravenous administration of N-methyl nitrogen mustard, $CH_3N(CH_2CH_2Cl)_2$, could result in human tumor regression, Rhoads went in search of funding to develop this experimental treatment known today as chemotherapy.[4] He persuaded Alfred P. Sloan Jr., the chairman of the General Motors company, along with the company's chief engineer, Charles F. Kettering, to endow a new institute that would bring together leading scientists and physicians to make a concerted attack on cancer.[4] On August 7, 1945, ironically the same day an atom bomb was dropped on Japan, they announced their plans for the Sloan Kettering Institute for Cancer Research—"World War II was over, but the war on cancer had just been launched,"[4] and relaunched, as it were, by President Nixon in 1971.

The above-mentioned beneficial structural change from $S(CH_2CH_2Cl)_2$, a chemical warfare agent, to $CH_3N(CH_2CH_2Cl)_2$, an anticancer agent, led to the con-

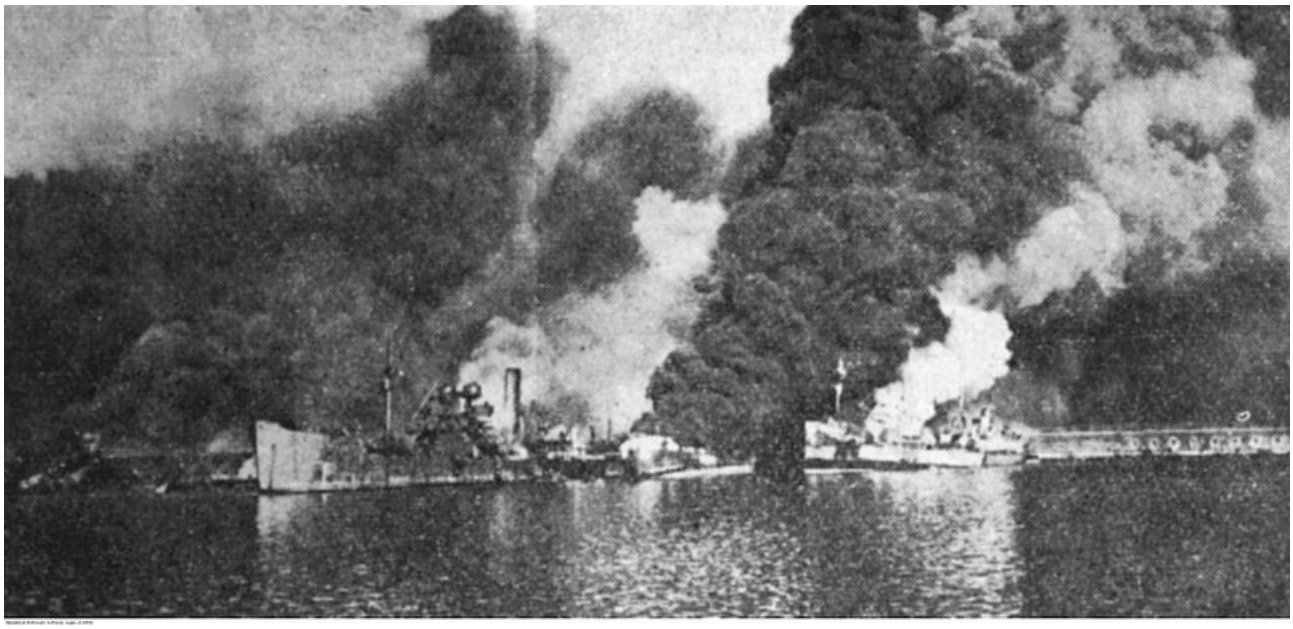


Figure 2. Photo of burning ships resulting from a German air force strike against the Anglo-American fleet in Bari, Italy, December 2, 1943, and free to use (public domain). A massive explosion of the SS *John Harvey*, a U.S. World War II Liberty ship released 130,000 pounds of "mustard gas," S(CH2CH2Cl)$_2$. A total of 628 military victims were hospitalized with mustard gas symptoms, and by the end of the month, 83 of them had died. The number of civilian casualties, thought to have been even greater, could not be accurately gauged since most had evacuated the city to seek shelter with relatives.

cept of analogous RN(CH_2CH_2Cl)$_2$ structures wherein the chemical nature of R could be varied to modulate CH_2CH_2Cl alkylating activity, i.e. $CH_2CH_2Cl \rightarrow CH_2CH_2Nuc$, Nuc = nucleophile, e.g. N or O in DNA. The use of phosphorus attached to nitrogen as a possible R group in RN(CH_2CH_2Cl)$_2$ for modulating DNA alkylating activity, and hence DNA crosslinking potential by virtue of having two CH_2CH_2Cl alkylating moieties, was rationalized as follows.

In a 1954 publication in *J Amer Chem Soc*, Orrie M. Friedman (Brandeis University) and Arnold M. Seligman (Harvard Medical School) stated[6] that they "considered it worthwhile to re-examine the question of the abundance of phosphamidase activity in malignant tissue as compared to normal tissues, with substrates which could be used as possible chemotherapeutic agents," should earlier reports of higher enzymatic activity "represent the true state of affairs." They reasoned that "phosphorylated nitrogen mustards would be expected to be devoid of mustard action as a consequence of loss of basicity of the nitrogen atom in the phosphamide bond, [and] enzymatic hydrolysis of this bond would liberate nitrogen mustard within cells in proportion to their phosphamidase activity." In this envisioned phosphorylated version of RN(CH_2CH_2Cl)$_2$, R = P(O)XY with X and Y being variable substituents. Consequently, they further reasoned, "[i]f malignant cells were, indeed, rich in phosphamidase activity, more nitrogen mustard could be delivered to them by the intravenous injection of a suitable N-phosphorylated nitrogen mustard than by injection of a tolerated dose of nitrogen mustard itself."[7] Of the synthesized phosphorylated bis-(2-chloroethyl)amines in which at least one of the groups on phosphorus is either amino or hydroxyl, there was extensive spontaneous hydrolysis of the reported analogs of PM (Figure 1) over the physiologically relevant pH range 4.5-8.5.[6] Later in 1954, Friedman and coworkers reported that N-phosphorylated secondary nitrogen mustards, including PM, were "capable of intramolecular cyclization to potent tertiary amine mustards," (i.e. highly reactive 3-membered ring aziridinium ions to be discussed in another section) and "were much less toxic" than nitrogen mustard.[8] They concluded by suggesting that "[s]uitable phosphoryl derivatives of the new secondary nitrogen mustards may turn out to be chemotherapeutic agents for tumors with high phosphamidase activity."[8]

More or less concurrent with the above synthetic work and suggestive reasoning in the U.S.,[6, 8] Norbert Brock and coworkers at Asta-Werke Aktiengesellschaft Chemische Fabrik, Brackwede, Germany, published[9] a 1958 paper in German titled (when translated to Eng-

United States Patent Office 3,018,302 Patented Jan. 23, 1962

3,018,302

NOVEL CYCLIC PHOSPHORIC ACID ESTER AMIDES, AND THE PRODUCTION THEREOF

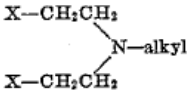
Herbert Arnold, Bielefeld, Norbert Brock, Wadersloh, and Friedrich Bourseaux, Bielefeld, Germany, assignors to Asta-Werke Aktiengesellschaft Chemische Fabrik, Brackwede, Germany

No Drawing. Filed Apr. 15, 1958, Ser. No. 728,568
Claims priority, application Germany Feb. 10, 1956
9 Claims. (Cl. 260—461)

This invention relates to novel cyclic phosphoric acid ester amides, and the production thereof.

This application is a continuation-in-part of our copending patent application Serial No. 638,235, filed February 5, 1957, entitled "Novel cyclic phosphoric acid ester amides and the production thereof."

For a number of years, compounds of the type of the so-called nitrogen mustard gas which conform to the following formula

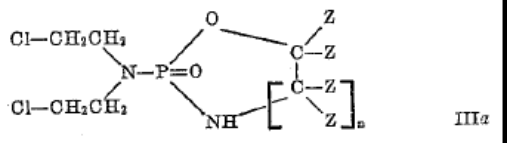


I

in which X is a halogen atom, preferably a chlorine atom, have found considerable medical interest for the treatment of malignant growths and similar diseases owing to their inhibiting action on the splitting up of cells. In this connection, chemical and pharmacological works have been published concerning the synthesis and effective mechanism of different derivatives of nitrogen mustard gas, which have as a common feature the direct linking of the nitrogen atom to other groups.

intended to comprise alkyl radicals with straight and branched chains.

The compounds of the general Formula III which are preferred according to the invention correspond to the following general formula



IIIa

wherein Z and *n* have the same meaning in Formula III.

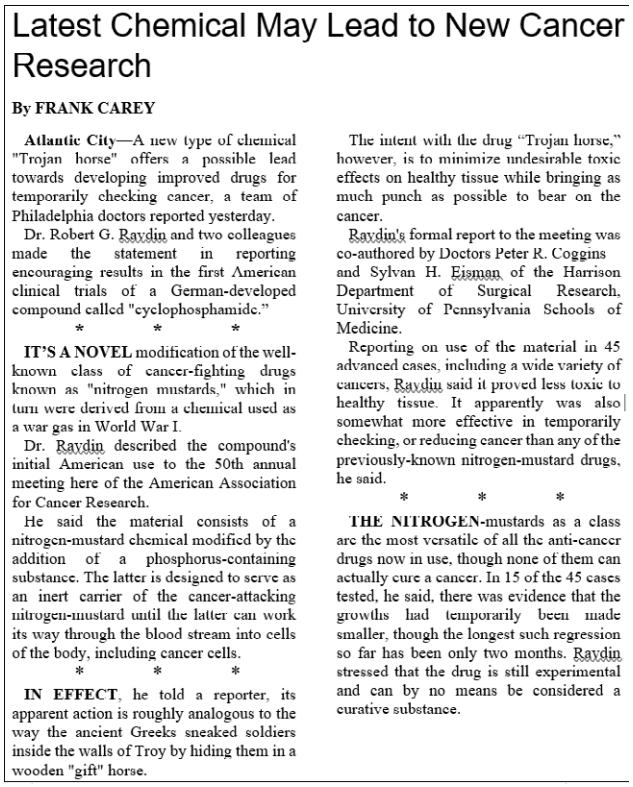
Preferably at least two Z's stand for hydrogen, whereas the remaining Z's have the same meaning as in Formula III.

The products of the general Formulae III and IIIa have a particularly favourable therapeutic effect as cytostatics that is to say as agents inhibiting cell growth in the treatment of malignant growths and similar diseases. The products were applied in therapeutic tests using the Yoshida ascitic tumour, the Jensen's sarcoma and the Walker carcinoma. With the Yoshida ascitic tumor the lethal and the curative doses (DL_{50} and DC_{50} values) shown in the following table were ascertained. The criterion of the curative doses (DC) effect was a cure in 90 days. The curative doses were applied in four partial doses, whereas the lethal doses were applied in one single dose. The following table shows in the second vertical column the partial curative dose i.e. one fourth of the total curative dose and in the third vertical column the approximate total lethal dose.

Figure 3. Image of the face page of U.S. Patent 3,018,302 issued to Arnold et al. at Asta-Werke Aktiengesellschaft Chemische Fabrik, Brackwede, Germany covering the synthesis and anticancer properties of CP (n = 2, Z = H) and other cyclic analogs (see boxed text).

lish) *Novel cancer chemotherapeutic agents from the group of cyclic nitrogen mustard phosphamide esters*, wherein results for CP were described. Brock has said[3] that he and his team had synthesized and screened more than 1,000 of these candidate oxazaphosphorine compounds. Synthetic details for CP and other cyclic analogs were later available in a U.S. issued patent (3,018,302) published in 1962 by Arnold et al. at Asta-Werke (Figure 3). In this patent, exemplary data are given which show CP as having the most promising therapeutic index (TI) *in vivo* in several model tumor systems, commonly used to measure a drug's safety. This index, with the formula TI = TD_{50}/ED_{50} was (and still is) commonly used to measure a drug's safety "window," as TD_{50} is the median toxic dose while ED_{50} is the median effective dose.

This patented discovery of promising *in vivo* antitumor activity for CP in animal models described by Arnold et al. led to early clinical trials in Germany[10] and the U.S. A noteworthy historical newspaper account of the latter, which was published on April 13, 1959, is reproduced in Figure 4. This newspaper article reports on "encouraging results in the first American clinical trials of a German-developed compound called 'cyclophosphamide'," which were described by Dr. Robert G. Ravdin (Department of Surgical Research, University of Pennsylvania Schools of Medicine) at the 50th annual meeting of the American Association for Cancer Research held in Atlantic City, New Jersey. The newspaper article goes on to say that this trial involved administration of CP to 45 patients with a wide variety of



Latest Chemical May Lead to New Cancer Research

By FRANK CAREY

Atlantic City—A new type of chemical "Trojan horse" offers a possible lead towards developing improved drugs for temporarily checking cancer, a team of Philadelphia doctors reported yesterday.

Dr. Robert G. Raydin and two colleagues made the statement in reporting encouraging results in the first American clinical trials of a German-developed compound called "cyclophosphamide."

* * *

IT'S A NOVEL modification of the well-known class of cancer-fighting drugs known as "nitrogen mustards," which in turn were derived from a chemical used as a war gas in World War I.

Dr. Raydin described the compound's initial American use to the 50th annual meeting here of the American Association for Cancer Research.

He said the material consists of a nitrogen-mustard chemical modified by the addition of a phosphorus-containing substance. The latter is designed to serve as an inert carrier of the cancer-attacking nitrogen-mustard until the latter can work its way through the blood stream into cells of the body, including cancer cells.

* * *

IN EFFECT, he told a reporter, its apparent action is roughly analogous to the way the ancient Greeks sneaked soldiers inside the walls of Troy by hiding them in a wooden "gift" horse.

The intent with the drug "Trojan horse," however, is to minimize undesirable toxic effects on healthy tissue while bringing as much punch as possible to bear on the cancer.

Raydin's formal report to the meeting was co-authored by Doctors Peter R. Coggins and Sylvan H. Eisman of the Harrison Department of Surgical Research, University of Pennsylvania Schools of Medicine.

Reporting on use of the material in 45 advanced cases, including a wide variety of cancers, Raydin said it proved less toxic to healthy tissue. It apparently was also somewhat more effective in temporarily checking, or reducing cancer than any of the previously-known nitrogen-mustard drugs, he said.

* * *

THE NITROGEN-mustards as a class are the most versatile of all the anti-cancer drugs now in use, though none of them can actually cure a cancer. In 15 of the 45 cases tested, he said, there was evidence that the growths had temporarily been made smaller, though the longest such regression so far has been only two months. Raydin stressed that the drug is still experimental and can by no means be considered a curative substance.

Figure 4. Typed facsimile of an April 13, 1959, article in the *Utica Daily Press* (Utica, New York, U.S.) found by the author (G. Z.) in an open-source digital archive of newspapers in the U.S. (The Fulton History Newspaper Site). The article by Frank Carey reports on "encouraging results in the first American clinical trials of a German-developed compound called 'cyclophosphamide,'" which were described by Dr. Robert G. Ravdin at the 50th annual meeting of the American Association for Cancer Research held in Atlantic City, New Jersey. Ravdin's formal report at the meeting was co-authored by Drs. Peter R. Coggins and Sylvan H. Eisman of the Harrison Department of Surgical Research, University of Pennsylvania Schools of Medicine. The typed facsimile prepared by the author (G. Z.) was necessary due to the poor quality of the digitalized optical character reading of the original newspaper article.

advanced cancers, and that "Ravdin said CP proved less toxic to healthy tissue, and apparently was also somewhat more effective in temporarily checking, or reducing cancer than any of the previously-known nitrogen-mustard drugs." Ravdin added that CP's "apparent action is roughly analogous to the way the ancient Greeks sneaked soldiers inside the walls of Troy by hiding them in a wooden 'gift' horse. The intent with the drug 'Trojan horse,' however, is to minimize undesirable toxic effects on healthy tissue while bringing as much punch as possible to bear on the cancer."

A full report on the outcome of treating additional cancer patients with CP in this landmark study in the U.S. was published by Coggins, Ravdin and Eisman in 1960.[11] By December 29, 1959, the time their manuscript was submitted for publication, a total of 143 patients with terminal cancer had received CP therapy, of whom 82 were considered to be evaluable, i.e. showing either objective progression or regression of cancer. Twenty-nine of these evaluable patients showed definite regression of their disease, which is ~35% efficacy and quite good, considering that the patients selected for treatment with CP were judged to have had terminal cancer.

As will be evident from the next section on metabolism of CP, the 'Trojan horse' analogy attributed to Ravdin in the newspaper article in Figure 4 turned out to be conceptually correct, in a general sense, but actually involves chemistry and enzymes quite different from those proposed in the original ideas about activation of CP by a phosphamidase in cancer cells.[6] In any event, these and additional early clinical findings led to the above-mentioned approval of CP for treatment of lymphoma by the U.S. FDA in 1959.

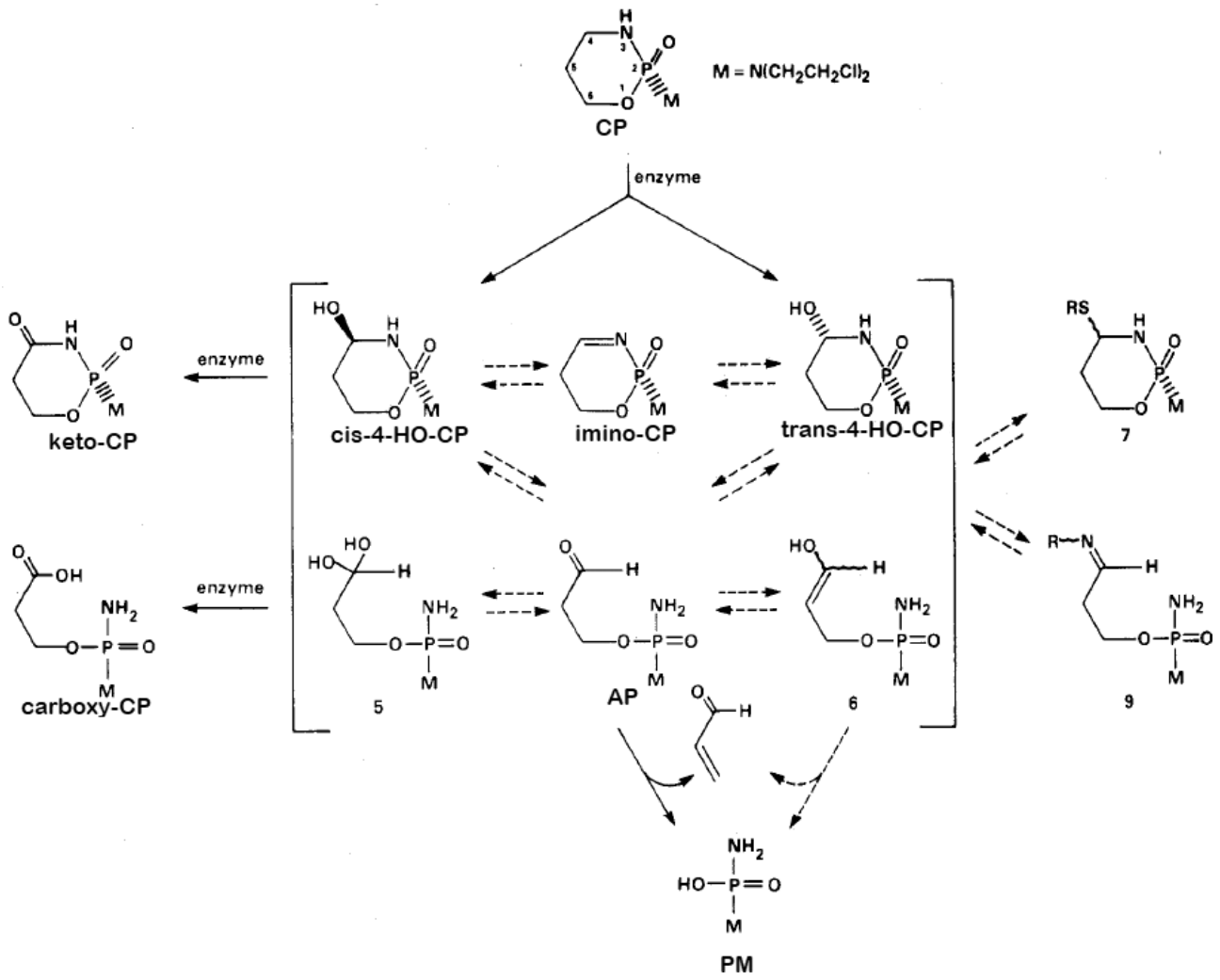
As a final comment to this early historical background, Ravdin's 1959 use of the 'Trojan horse' analogy (Figure 4) for release of PM in cancer cells was quite unique in that the author (G. Z.) was unable to find any articles in Google Scholar published between 1940-1959 with the term 'Trojan horse' associated with drug design, delivery, or similar terms. Interestingly, 1959 was also the year in which the term "prodrug" was first introduced by the Parke-Davis company for chemical modification of the chloramphenicol structure in order to improve this antibiotic's bitter taste and poor solubility in water. While the term prodrug is now universally applicable in modern medicinal chemistry, Ravdin's 'Trojan horse' descriptor is historically appealing.

METABOLISM OF CP

Mean rate of absorption of CP after oral administration from studies in which the drug was given in a high dose both orally and intravenously to the same tumor patients (n = 18) is very high (~88%), which indicates that CP may be administered orally with good bioavailability at that high dose.[12] The pharmacokinetics of CP in patients (n = 7) with various types of cancer has been characterized by a two-compartment model wherein the half-life of the elimination phase of CP ranged between 3 and 11 hours.[13] The calculated fraction of the dose of CP which was metabolized averaged 88%.[13]

According to Brock,[3] CP and other oxazaphosphorine cytostatics differ from directly alkylating compounds in that "they must undergo biotransformation

Scheme 1[a,b]



[a] M = $N(CH_2CH_2Cl)_2$

[b] Adapted with permission from *J Med Chem* **1987**, *30*, 366–374. Copyright 1987 American Chemical Society.

before they can exert their alkylating oncocidal action." Deciphering the metabolic pathway for this "activation" of CP *in vivo* and identifying CP metabolites (Scheme 1) evolved into subjects of considerable clinical interest and extensive investigations by many research groups. In 1970, Hill et al.[14] reported the isolation and identification of 4-ketocyclophosphamide (keto-CP, Scheme 1) from urine of dogs given CP as "a possible active form" of CP. One year later in 1971, Bakke et al.[15] similarly analyzed sheep urine and identified a ring-opened carboxylated metabolite (carboxy-CP, Scheme 1) in addition to keto-CP. Shortly thereafter, in 1973, Colvin et al.[16] reported the isolation of PM from incubations of CP with mouse liver microsomes, using mass spectrometry (MS) for identification, and suggested that "[t]his compound may play a major role in the biological activities" of CP.

The following year in 1974, Connors et al.[17] at the Chester Beatty Research Institute, Royal Cancer Hospital, London, England, reported enlightening findings from a comprehensive study of microsomal metabolism of CP in the liver wherein CP is first converted, "presumably by the mixed-function oxidases," into 4-hydroxycyclophosphamide (4-HO-CP, Scheme 1), "which may then break down by elimination of acrolein [H_2C=CHC(O)H, Scheme 1] from its tautomeric form, aldophosphamide" (AP, Scheme 1) to yield PM. In competition with this process is the enzymic conversion of 4-HO-CP (by dehydrogenation) and AP (by oxidation) into the known *in vivo* metabolites of CP, keto-

CP and carboxy-CP, respectively, "each of which has low cytotoxicity." Connors et al.[17] noted that 4-HO-CP, "which was too unstable to allow identification directly by conventional procedures, was trapped by reaction with ethanol." Importantly, however, these investigators found that the resulting two, apparently isomeric, ethyl derivatives, (1) were amenable to MS, (2) yielded acrolein 2,4-dinitrophenylhydrazone on treatment with acidic 2,4-dinitrophenylhydrazine, (3) were hydrolyzed in water (pH 4.3), each isomer apparently regenerating 4-HO-CP, and (4) were highly toxic to Walker tumor cells in culture. PM was also isolated after *in vitro* metabolism of CP, and on the basis of a bioassay involving Walker tumor cells in whole animals it was found that, of the known metabolites of CP, only PM "possesses the cytotoxicity and biological half-life appropriate to the active antitumor metabolite."[17]

Later, in 1977, Fenselau et al.[18] provided additional MS data for AP as a transient intermediate in the metabolism of CP by means of the isolation and characterization of the cyanohydrin derivative of AP from incubation of CP with mouse liver microsomes in the presence of appropriate aldehyde trapping reagents, namely, sodium bisulfite followed by sodium cyanide. Moreover, AP was also identified in the plasma of a patient receiving CP, after treatment of the plasma with these trapping reagents. As a cautionary final comment, Fenselau et al.[18] stated that the ethanol trapping data of Connors et al.,[17] mentioned above, "might alternatively arise from addition of ethanol across the double bond" of a putative iminocyclophosphamide (imino-CP, Scheme 1) intermediate, which was apparently the first mention of this possibly new metabolite that is uniquely characterized by a presumably reactive C=N double bond conjugated with a phosphoryl (P=O) moiety, i.e. [C=N-P=O ↔ $^{+}$C-N=P-O^{-}].

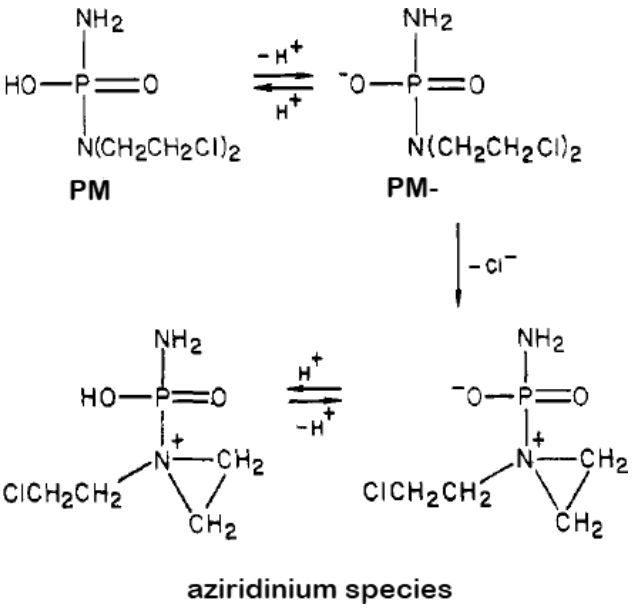
Better understanding of the basic biochemistry and interrelationships of all of the above-mentioned CP metabolites, especially the postulated reactive imino-CP metabolite, are discussed in the following sections, which involve use of highly informative multinuclear NMR methodology.

NMR SPECTROSCOPIC ELUCIDATION OF CP METABOLITES AND KINETICS

Application of multinuclear (^{31}P, ^{13}C, ^{2}H and ^{1}H) Fourier-transform NMR spectroscopy was integral to my various NCI-funded research projects related to CP and its metabolism, which had been previously studied by others largely using MS. Important advantages of NMR over MS include (1) data-rich structural details (e.g. chemical shift, coupling constants, and spin decoupling), (2) real-time data acquisition, and (3) inherent molar quantification, all of which combine to provide (4) unambiguous molecular identification, while (5) temperature control enables (6) direct measurement of molecular dynamic processes and/or (7) kinetics.

Initially, ^{31}P-NMR was used to evaluate the influence of pH on the rate of intramolecular cyclization of a NCH_2CH_2Cl moiety to a 3-membered ring aziridinium ion and the hydrolysis of this reactive alkylator. While an influence of pH on the alkylating activity of PM is expected based on first-principles of organic chemistry, no data was available at the time. Briefly, as we reported in 1979 (Engle et al.[19]), the ^{31}P- NMR kinetic data for PM demonstrated that the half-life of this metabolite of CP exhibits appreciable variation (~5-fold decrease) over the physiologically relevant pH range of 6-8, and that the anionic conjugate base of PM (PM-) is the required precursor to its intramolecularly cyclized aziridinium ion species (Scheme 2). The relative concentration of this aziridinium ion reached a peak that was also dependent on pH, increasing with lower pH. It was therefore suggested[19] that pH control over the concentration of PM- and the rate of its intramolecular cyclization, as well as the rates of reaction of the cyclized aziridinium ions with nucleophiles, "provides a chemical basis for rationalizing at least part of the oncostatic specificity" of CP.

Scheme 2[a]



[a] Adapted with permission from *J Med Chem* ***1979****, 22,* 897-899. Copyright 1979 American Chemical Society.

The rate for cyclization of PM- to its aziridinium ion would be ~50% slower in tumor cells that may be more acidic than normal cells (pH 6.9 vs. 7.4)[20] and, after the active aziridinium alkylator is generated, it will have a longer lifetime under relatively acidic conditions, due to less frequent interception by hydroxide ion. Whether alone or in concert, these circumstances provide for greater probability of encountering and alkylating DNA and, in effect, represent a form of selective (i.e. pH-dependent) cross-linking. A full paper on this subject with additional experiments was published by Engle et al. 1982.[21]

During our NMR investigations in the late 1970s and early 1980s, a detailed understanding of the multifaceted mechanism of selective cytotoxicity had to contend with a formidable array of intervening chemistry (Scheme 1) that was poorly understood for the presumed interconversion of *cis*-4-HO-CP, AP, and *trans*-4-HO-CP, and had several open questions. For example, the kinetics and thermodynamics of these presumed equilibria had not been measured, although circumstantial evidence had led to the suggestions[22] that AP is much less stable than 4-HO-CP and that no equilibrium exists between 4-HO-CP and AP at room temperature or above. Similarly, there was no conclusive information regarding possible equilibria between the aldehyde moiety in AP, its hydrate **5**[22] and its enol **6**[23] or equilibria between *cis*-4-HO-CP and *trans*-HO-CP. A possible equilibrium between AP and **5** was more than academically interesting when one considers that *in vivo* factors that might influence the kinetics and thermodynamics of the AP hydration to **5** could modulate, in effect, the location and rate of release of PM and acrolein from AP. This point was perhaps more apparent in the context of sulfhydryl compounds that were reported[24-25] to "deactivate" and transport 4-HO-CP/AP by the reversible formation of 4-thiocyclophosphamide conjugates (**7**). Also, imino-CP had been reportedly identified[26] and then criticized[27] as a possible intermediate in these chemical transformations. Furthermore, we reasoned that if imino-CP was actually an intermediate, there could be bimolecular counterparts of imino-CP, namely, Schiff-base conjugates of AP. All of these reversible processes, as well as the irreversible fragmentation of AP (or **6**[23]) into acrolein and PM, and the alkylation chemistry of PM/PM- would be influenced by pH and metal ions. While data for all this complex chemistry was sorely lacking, information had been reported[28] for the enzymatic "detoxification" of 4-HO-CP/AP that produces the urinary metabolites keto-CP and carboxy-CP.

With the aim of clarifying the foregoing chemistry issues and open questions, we used known synthetic methods to prepare *cis*-4-hydroperoxycyclophosphamide (*cis*-4-HO_2-CP) as well as its 5,5-dideuterio (D = ^{2}H) and 4-^{13}C isotopically labeled versions to allow unambiguous assignment of NMR signals to CP metabolites. This via initial reductive conversion of *cis*-4-HO_2-CP to *cis*-4-HO-CP with sodium thiosulfate ($Na_2S_2O_3$) in 2,6-dimethylpyridine (lutidine) buffer at pH 7.4, 37 °C. Full details and the results obtained were published[29] in 1984. Some of the salient findings and implications are briefly summarized as follows.

The stereospecific deoxygenation of synthetic *cis*-4-HO_2-CP with 4 equivalents of $Na_2S_2O_3$ afforded, after ~20 min, a "pseudoequilibrium" distribution of *cis*-4-HO-CP, AP, hydrated AP, **5** (Scheme 1), and *trans*-4-HO-CP in the relative proportions of 57:4:9:30, respectively, which remained constant during their continual disappearance by irreversible reactions. NMR signals indicative of imino-CP and enol **6** (Scheme 1) were not detected (<0.5-1% of the synthetic metabolite mixture). A computerized least-squares fitting procedure was applied to the individual ^{31}P-NMR derived time-courses for conversion of *cis*-4-HO-CP, AP plus AP hydrate ("AP"), and *trans*-4-HO-CP into acrolein and PM, the latter of which gave an expected array of thiosulfate S-alkylation products and other phosphorus-containing materials derived from secondary decomposition reactions. This kinetic analysis gave the individual forward and reverse rate constants for the apparent tautomerization processes, i.e. *cis*-4-HO-CP ↔ "AP" ↔ *trans*-4-HO-CP, as well as the rate constant (k_{AP}) for the irreversible fragmentation of AP. Replacement of the $HC(O)CH_2$ moiety in AP with $HC(O)CD_2$ led to a primary kinetic isotope effect (k_H/k_D = 5.6 ± 0.4) for k_{AP}, consistent with a primary effect for rate-determining removal of a proton that is adjacent to a carbonyl group. The apparent half-lives ($t_{1/2}$) for *cis*-4-HO-CP, "AP", and *trans*-4-HO-CP, under the above reaction conditions, were each equal to ~38 min, which is considerably shorter than the then widely cited colorimetrically derived half-lives reported by earlier investigators.[30]

Next, *N*-acetyl-L-cysteine (R*SH) was used to study its conversion of 4-HO-CP/AP to C4-SR* conjugates of the type previously reported by Hohorst et al.[25] In brief, detailed analysis of the resultant complex ^{31}P-NMR spectra supported the earlier conclusions by by Hohorst et al.[25] In the presence of the commonly used buffer tris(hydroxymethyl)aminomethane (Tris) at pH 7.4, 37 °C, AP gave rise to a ^{31}P-NMR signal that was unambiguously identified as an aminal adduct, initiated by reaction of AP's C(O)H moiety and the NH_2 group of Tris. Other investigators[31] had mistakenly ascribed this ^{31}P-NMR signal to 4-HO-CP. Final-

ly, ^{13}C- and ^{2}H-NMR studies of the decomposition of 5,5-dideuterio and 4-^{13}C isotopically labeled versions of *cis*-4-HO-CP revealed, via spectra of the isotopically labeled acrolein fragments [$H^{13}C(O)CH=CH_2$ and $HC(O)CD=CH_2$], previously unrecognized chemical complexities. Namely, essentially all of this urotoxic metabolite was in rapid, reversible equilibrium with thermodynamically favored adducts at pH 7.4, 37 °C. This represented a caveat for metabolic and toxicological investigations of the "acrolein" metabolite produced by fragmentation of AP inasmuch as *in vivo* there may be essentially no free $HC(O)CH=CH_2$ *per se*.

As was mentioned above, there was no NMR evidence for detectable amounts of imino-CP,[29] which Fenselau et al.[26] had earlier proposed as a novel, chemically reactive metabolite of CP, as did Borsch et al.[32] While the inability to detect imino-CP by NMR does not preclude its existence, it does suggest that, under the conditions of our reported[29] studies, the concentration of imino-CP, if formed, is quite low. In order to optimize the conditions under which an imino oxazaphosphorine analog of imino-CP might form and be detected by NMR, I reasoned that possible addition and fragmentation pathways would have to be controlled. This led to the synthesis of 4-hydroxy-5,5-dimethylcyclophosphamide for NMR studies in a nonnucleophilic solvent, which we reported in 1987 (Boyd et al.[33]). Like 4-HO-CP, this 5,5-dimethyl analog of CP can undergo ring-opening and dehydration reactions, thus affording the 5,5-dimethyl counterparts of AP and imino-CP, respectively; however, the absence of a C-5 proton blocks the formation of PM by an α,β-elimination mechanism. Characterization of the 5,5-dimethyl analog of AP would then provide NMR spectral benchmarks applicable to imino-CP. Using reduction of a corresponding *cis*-4-hydroperoxy precursor, as was mentioned above for CP,[29] and anhydrous dimethyl sulfoxide (DMSO) solvent, it was possible to detect ^{1}H, ^{13}C, and ^{31}P chemical shifts in NMR spectra that were unambiguously ascribed to 5,5-dimethyl imino-CP.[33] In DMSO solution, concentrations of the dimethyl analogs of *cis-/trans*-4-HO-CP, AP, and imino-CP were found to be temperature-dependent with higher temperatures favoring aldehydic and imino analogs in a reversible manner, thus indicating that dimethyl *cis-/trans*-4-HO-CP, AP, and imino-CP were interconverting. Once the spectral characteristics of 5,5-dimethyl imino-CP were thus identified, they were used as benchmarks to locate the elusive imino-CP of the parent CP. Repeating the experiment with 4-hydroperoxy-CP led to the observation of authentic imino-CP. The addition of small amounts of water, a nucleophile, to DMSO solutions of imino-CP resulted in the immediate disappearance of its NMR signals. Thus, formation of any imino-CP *in vivo* is expected to lead to rapid conjugation reactions with biological nucleophilic species.

^{31}P-NMR SPECTROSCOPIC OBSERVATION OF THE INTRACELLULAR TRANSFORMATIONS OF CP METABOLITES

In the early 1980s, Jack S. Cohen and coworkers at the NIH pioneered metabolic studies of mammalian cells by ^{31}P-NMR using a continuous perfusion technique wherein viable cells were embedded in a matrix of agarose gel in the form of fine threads which were continuously perfused in a standard NMR tube.[34] The small diameter of the thread allows rapid diffusion of metabolites and drugs into the cells. The changes in ^{31}P-NMR signals, exemplified with ATP and P_i levels, were followed as a function of time in response to perfusion with a glucose-containing medium, with isotonic saline and with a medium containing 2,4-dinitrophenol, an uncoupler of oxidative phosphorylation.[34] These researchers suggested[34] that "[t]his gel-thread perfusion method should enable routine NMR studies of cellular metabolism, and may have other potential biological applications."

Around this time, I had already moved from CUA to the FDA Division of Biochemistry and Biophysics located on the NIH campus, and collaborated with my FDA colleague, William Egan, on the CP metabolite NMR investigations mentioned above.[19, 29, 33] Since Egan had earlier worked with Cohen at NIH, he quite naturally became interested in applying Cohen's cell/gel-perfusion method to CP metabolites. The salient results of Egan's pursuit of such studies are briefly summarized as follows, and were reported[35] in full detail in *J Med Chem* in 1986.

^{31}P-NMR spectroscopy was used to directly monitor, for the first time, the intracellular chemistry of the ultimate active metabolite of CP, namely, PM. These NMR studies utilized a human histiocytic lymphoma cell line (U937), embedded in agarose gel threads contained in a 10-mm NMR tube, as described by Cohen and coworkers.[34] The cell/gel treads were perfused with medium containing a mixture of synthetically derived CP metabolites, namely, 4-HO-CP, AP, and PM (via reduction of *cis*-4-HO_2-CP in a separate solution). As can be seen from the time course of the ^{31}P-NMR spectra (a-f) shown in Figure 5, the 4-HO-CP and/or AP metabolites readily crossed the cell membrane, and the increasing intracellular concentration of PM could, therefore, be

attributed primarily to the intracellular fragmentation of AP. Signals suggestive of either carboxy-CP or keto-CP were not detected. In companion experiments, there was no measurable cellular uptake of PM, presumably due to its anionic character at physiological pH and the hydrophobic nature of cell membrane lipid bilayers.

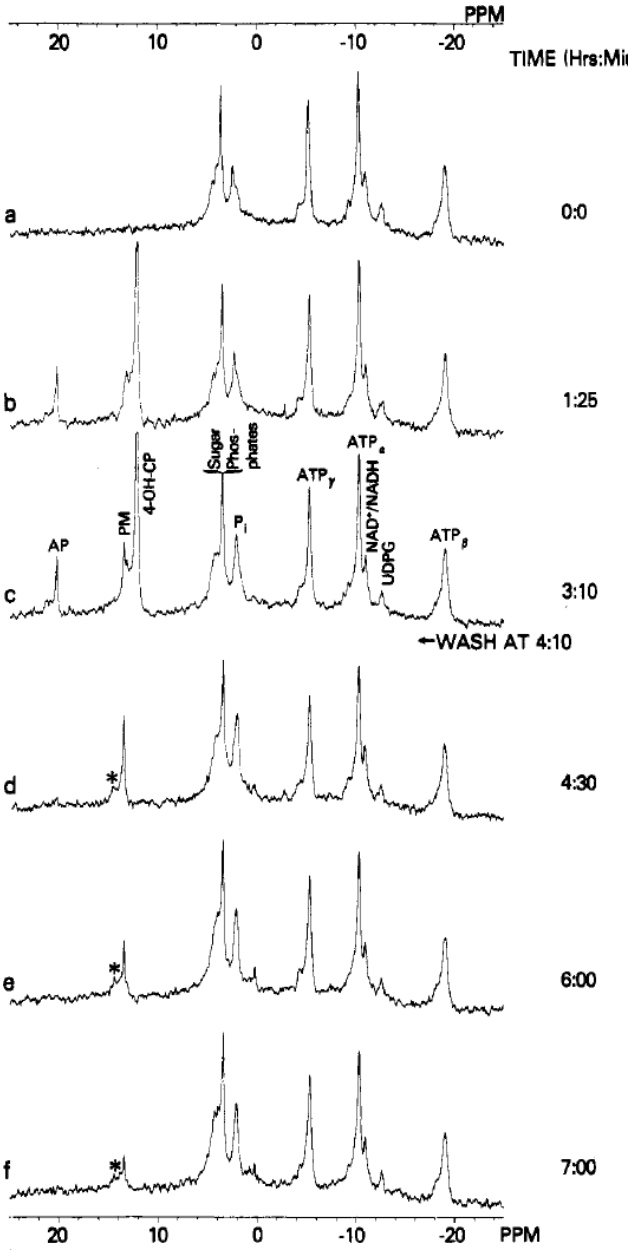


Figure 5. ^{31}P-NMR spectra (161 MHz) of U937 cells as a function of time and in the presence of added 4-HO-CP and after washing cells with fresh perfusate. Abbreviations: AP = aldophosphamide, PM = phosphoramide mustard, 4-OH-CP = 4-hydroxy cyclophosphamide, P_i = inorganic phosphate, ATP = adenosine triphosphate, NAD^+/NADH = oxidized and reduced nicotine adenine dinucleotide, UDPG = uridine diphosphoglucose (galactose). An asterisk (*) denotes an unknown impurity (which was not observed in other runs). Reproduced with permission from *J Med Chem* ***1986****, 29, 1206-1210.* Copyright 1986 American Chemical Society.

Spectral data were used to calculate a rate constant for the intracellular disappearance of PM at 23 °C. The intracellular pH was determined to be 7.1 from the chemical shift of the internal inorganic phosphate signal. The intracellular disappearance of PM (see Figure 5 d-f) followed a first-order decay law. Least-squares fitting of the intracellular concentration of PM, as a function of time, to a simple first-order process provided a half-life ($t_{1/2}$) of 125 min at 23 °C. Considering variations in conditions, this intracellular half-life of 125 min compares favorably with $t_{1/2}$ of 48 min reported by Voelcker et al.[36] for PM in phosphate buffer at pH 7.0, 37 °C; allowing a factor of ~2 in rate for every 10 degrees in temperature, the adjusted half-lives are essentially the same. The value for the half-life in Tris buffer at pH 7.0, 37 °C determined by Engle et al.[19] is somewhat short (14 min) relative to that found intracellularly, indicating that medium effects can be moderately significant.

In concluding this section on the first-ever demonstration of the use of NMR to study CP and its metabolites in living cells, it is worth noting that subsequent advances in NMR spectroscopy led to the design of surface coils to allow *in vivo* detection of NMR signals of molecules that are present within the magnetic fields in tissue and/or blood vessels in proximity to the surface coil. The feasibility of this approach for CP was first reported in 2000 by Payne et al.[37] at the CRC Clinical Magnetic Resonance Research Group in Surrey, UK, who detected ^{31}P-NMR signals from CP in the livers of patients *in vivo*. The signals were sufficiently large that they may be detected using simple pulse-and-acquire measurements employing a relatively small (8-cm diameter) ^{31}P surface coil. The use of ^{1}H-decoupling yielded substantial (~4-fold) improvement in sensitivity. In terms of nomenclature, following the development of NMR imaging using ^{31}P or other non-radioactive nuclei, the preferred terminology was simplified from nuclear magnetic resonance (NMR) spectroscopy to magnetic resonance spectroscopy (MRS), thereby eliminating the "scary" (to non-scientists) word nuclear, and also differentiating MRS from signal-acquisition methods that are based on radioactive isotopes, such as positron emission tomography (PET).

CP TUMOR CELL TOXICITY VS. NORMAL CELL DETOXIFICATION, AND RESISTANCE

The pharmacology of CP and its metabolites known in the 1970s was described by Connors et al.[17] in their

publication of studies of the active intermediates formed in the microsomal metabolism of CP. Briefly, it was stated that CP (cf. Scheme 1) is first converted, presumably by mixed-function oxidases (e.g. cytochrome P450), into 4-HO-CP, which may then fragment by elimination of acrolein from 4-HO-CP's tautomeric form, AP, to yield the known cytotoxic agent PM. This purely chemical fragmentation of AP into acrolein and PM competes with the enzymic conversions of 4-HO-CP (by dehydrogenation) and AP (by oxidation) into the known *in vivo* metabolites of CP, keto-CP and carboxy-CP, respectively, each of which has low cytotoxicity. Keto-CP and carboxy-CP are the principal urinary metabolites of CP, but are non-toxic to Walker tumor cells in a reported[17] bioassay system. In contrast, PM is markedly toxic and the dose needed to kill 75% of Walker tumor cells is low enough for it to be the toxic metabolite from "activated" CP. Acrolein, which has been detected following microsomal incubation of CP, had been suggested as the antitumor metabolite, but it is not as toxic as PM in this bioassay system.[17] Connors et al. add that the highly selective antitumor action of CP could be explained if normal cells, but not tumor cells, could efficiently convert the primary metabolites, 4-HO-CP and AP, by further enzymatic oxidation and dehydrogenation into the nontoxic keto-CP and carboxy-CP metabolites, respectively. In other words, "[t]umor cells would be selectively killed if they did not efficiently perform the detoxification process."[17] A proportion of the primary metabolites entering the tumor cell would break down spontaneously into the highly toxic PM and acrolein. The selective effect of CP could thus be due to intracellular release of PM specifically in tumor cells while the whole animal toxicity could be due to breakdown of the primary metabolite in extracellular fluid. This proposal[17] of cellular uptake of primary metabolites followed by intracellular release of PM were confirmed by the above-mentioned ^{31}P-NMR data obtained with cell/gel perfusion (Figure 5).[35]

Later, in 1994, ^{31}P-NMR was used to monitor in real-time the reaction of 4-HO-CP/AP with glutathione (GSH) – a known agent for drug conjugations – to form a mixture of four detectable diastereomers of 4-GSH-CP conjugates.[38] The 4-GSH-CP conjugates, which undergo the reverse reaction to reform 4-HO-CP/AP, were thus viewed as a "stabilized reservoir" of PM, which in turn can be intercepted (i.e. detoxified) by GSH through irreversible alkylation. This work was extended to show that levels of certain glutathione S-transferase isoenzymes in tumor cells can be related to development of resistance to CP.[39]

Along similar lines, about the same time, it was shown by the Sladek lab that class 1 and class 3 aldehyde dehydrogenases (ALDH-1 and ALDH-3, respectively) catalyze the detoxification of CP by metabolism of AP.[40] Thus, interindividual variation in the activity of either of these enzymes in, for example, breast cancers could contribute to the wide variation in clinical responses that are obtained when such regimens are used to treat these malignancies.[40] Perhaps the most compelling data to support the critical importance of ALDH in the metabolism of CP and the role it plays in the serendipitous history of this drug was published in 1996 by Magni et al.[41] Briefly, they tested whether ALDH-1 overexpression could directly induce CP resistance by cloning a full-length human ALDH-1 cDNA for retroviral vector transduction of CP-sensitive hematopoietic cell lines that were then tested for resistance to maphosphamide, a preactivated analog of CP. Overexpression of the ALDH-1 gene resulted in a significant increases in CP resistance, thus indicating that ALDH-1-mediated conversion of AP to non-toxic carboxy-CP is sufficient for cellular resistance to CP.

Additional insights into the detailed pharmacology of CP and its metabolites were also obtained from stereochemical studies that are described in the next two sections.

SYNTHESIS OF THE ENANTIOMERS OF CP

As mentioned in the introduction, my initial interest in CP in 1971 was in large part stimulated by wanting to investigate whether CP's chirality at phosphorus would influence its metabolism and anticancer activity, about which there were no publications to my knowledge. By 1975, I had used optically pure (+)-(*R*)-PhCHMeNH$_2$ to synthesize the two diastereomeric derivatives of CP having an N-CH(Me)Ph (N-α-methylbenzyl) moiety attached to the ring nitrogen.[42] Neither diastereomer underwent stereo-mutation at phosphorus when dissolved in human blood plasma at 37 °C for 14 h, or in H_2O-DMSO at pH ~5.6 or ~8.4 at 50 °C for 15 h, which indicated that enantiomerically pure CP would not racemize during its transport to the liver mixed-function oxidase hepatic system which effects C-4 hydroxylation to 4-HO-CP.[42] During the same time-frame, unbeknownst to me, Prof. Wojciech J. Stec and his students at the Polish Academy of Sciences Centre for Molecular and Macromolecular Studies in Lodz, Poland, had similar interests in CP stereochemistry, and likewise published in 1975 their synthesis of the same two diastereomeric N-α-methylbenzyl derivatives of CP.[43] Importantly, however, their publication also reported that the separated diastereomers underwent hydrogenolysis over Pd/C in EtOH to yield the individual enantiomers of CP.[43]

My lab soon thereafter carried out the same hydrogenolysis reaction to afford optically active (+)-CP that was proven by a novel NMR method[44] to be enantiomerically pure. Through fortunate circumstances, my CP collaborator William Egan (see above) was acquainted with chemist Jean Karle, whose mother, Isabella Karle was a noted crystallographer and the wife of crystallographer Jerome Karle, who would later be awarded the 1985 Nobel Prize in Chemistry with Herbert A. Hauptman for their outstanding achievements in the development of direct methods for the determination of crystal structures. The Karles were keen on collaborating with Egan and my lab to apply the then well-known "Karle and Karle" procedure[45] to determine the absolute configuration of phosphorus in (+)-CP without reference to a second asymmetric center of known chirality. After crystalline material was obtained, the crystal and molecular structure of enantiomerically homogeneous (+)-CP was determined by x-ray diffraction with the absolute configuration being established by the anomalous dispersion of the chlorine (Cl) and phosphorus (P) atoms (Figure 6).[46] It was found that the dextrorotatory (+) enantiomer of CP ([α]D/20 = 2.3 ° (*c* 3.0, methanol)) has the *R* configuration at P (*R*p). It was stated[46] that "the presently reported *R* configuration for (+)-CP provides a convenient and reliable basis for the establishment of the absolute configuration at phosphorus in all of the known chiral metabolites of CP [cf. Scheme 1] which may be synthesized from CP using reactions that do not involve stereochemical changes at the asymmetric phosphorus center." A footnote in this publication[46] reported preliminary *in vitro* kinetic measurements of liver microsomal "activation" of CP to give bis-(2-chloroethyl)amine (nor-HN2) wherein (-)-CP gave K_m = 0.57 mM and V_{max} = 27.4 μmol of nor-HN2 equiv g^{-1} h^{-1} and (+)-CP gave K_m = 0.48 mM and V_{max} = 22.2 μmol of nor-HN2 equiv g^{-1} h^{-1}. These Michaelis–Menten kinetic parameters implied that there was relatively little enzymatic discrimination between the (-)-CP and (+)-CP enantiomers, under these *in vitro* conditions. Additional findings later reported by others concerning the influence of CP stereochemistry on CP metabolism are given in the next section.

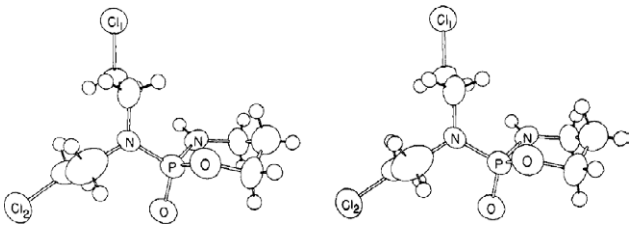


Figure 6. Stereodiagram of the absolute configuration of (+)-cyclophosphamide (CP). The ellipsoids representing the thermal parameters are at a 50% probability level. Hydrogen atoms are represented by small spheres. Reproduced with permission from *J Am Chem Soc* ***1977****, 99, 4803-4807.* Copyright 1977 American Chemical Society.

In the same year as our x-ray analysis of (+)-CP was published, Adamiak and Saenger at Abteilung Chemie, Max-Planck-Institut für experimentelle Medizin in Göttingen, Germany, in collaboration with the Stec lab in Lodz, Poland, reported[47] the results of an x-ray-diffraction study of (-)-CP that established the *S* absolute configuration at phosphorus (S_P), consistent with our independent finding R_P for (+)-CP.

EFFECTS OF STEREOCHEMISTRY ON CP METABOLISM IN HUMANS

The above-mentioned independent syntheses of the (-)-CP and (+)-CP enantiomers by Stec's laboratory and my group were quickly followed by a number of studies that compared the biological effects of the enantiomers with each other and with the racemate, the latter of which up to that time was the composition of CP administered to patients. The major question was whether evidence would be obtained indicating a substantially higher therapeutic index for either (+)-CP or (-)-CP compared to conventional racemic CP, (±)-CP.

The results obtained by my group were published[48] in 1979 and were comprised of *in vitro* metabolism studies and, through an NCI screening program, *in vivo* animal experiments. Briefly, separate incubation kinetic measurements for the metabolic "activation" of (+)-CP, (–)-CP, and (±)-CP by identical phenobarbital (PB)-induced mouse liver microsomal mixed-function oxidase preparations gave, respectively, V_{max} = 13.8 ± 1.0, 20.0 ± 1.5 and 16.3 ± 1.1 μmol nor-HN2 equiv g^{-1} h^{-1} and K_m = 0.37 ± 0.02, 0.56 ± 0.04 and 0.45 ± 0.02 mM. The absolute magnitude of the apparent V_{max} kinetic parameter increased by ~50% in a subsequent comparative run between (+)- and (–)-CP using a second preparation of the hepatic microsomal oxidase; however, the relative behavior of CP enantiomers toward enzymatic "activation" was constant, within experimental error, and revealed that V_{max}^{-}/V_{max}^{+} = 1.34 ± 0.17 and K_m^{-}/K_m^{+} = 1.35 ± 0.14. Removal and quantitative measurement, as a function of time, of free acrolein that is produced by incubation of CP with PB-induced microsomes repeatedly gave a roughly congruous family of "skewed bell-shaped" curves having maxima in the order (±)-CP > (+)-CP > (–)-CP; however, the differences between these acrolein time-course profiles were relatively small (~10–20%). Isolation of CP from separate (+)- and (–)-CP incubation mixtures, followed by determination of enan-

tiomeric homogeneity by NMR methods, demonstrated that CP is not racemized during *in vitro* liver microsomal metabolism. Mouse screening data (test/control percentages) for (+)-, (–)-, and (±)-CP activity against mouse L-1210 lymphoid leukemia showed no significant differences in therapeutic value. Collectively, these various experimental results suggested to us that "there is an unusually low degree of biological stereoselectivity associated with the metabolism of CP enantiomers."[48]

Stec's laboratory carried out far more comparative experiments of this sort *in vitro* and *in vivo* in several animal species, namely, mouse, rabbit, and rat, in collaboration with investigators at the Institute of Cancer Research, Surrey, England, as summarized in the introductory section of a 1979 publication (Jarman et al.[49]) that extended these collaborative studies to humans. The protocol for this small pilot study[49] involved 4 patients with carcinoma of the lung who had normal renal and hepatic function as measured by standard biochemical tests, and none had received CP previously. Doses (1 g i.v.) of (±)-CP, (+)-CP and (-)-CP were administered as a bolus, sequentially, with a 3-wk interval between doses. Blood samples were taken at 5, 10, and 30 min and at 1, 2, 4, 6, and 12 h. Urine was collected between 0 and 24 h after administration of the drug.

The salient results of this first-in-humans pilot study were summarized by Jarman et al.[49] as follows. The plasma levels of CP and the urinary output (24 h) of unchanged CP and of the two enzymatically produced metabolites, keto-CP and carboxy-CP (cf. Scheme 1), were determined using MS-stable isotope dilution. There was no significant difference between the three forms of CP with respect to plasma half-life (β phase) or in the urinary outputs of CP or carboxy-CP. The output of keto-CP after administration of (+)-CP was significantly greater than that produced from (±)-CP. CP recovered from the urine of patients given (±)-CP was either racemic or only slightly enriched in (-)-CP. The two enantiomers of CP were almost equally bound to plasma protein. Jarman et al.[49] concluded that "[b]ased on these metabolic studies alone, there is little reason to predict that the enantiomers will differ from each other or from the racemate in their therapeutic effects in humans, but there are other factors, e.g. , stereoselective uptake of the intermediary 4-hydroxylated metabolites [*cis*- and *trans*-4-HO-CP; cf. Scheme 1] by neoplastic cells, which could elicit such differences."

In concluding this section, it should be first mentioned that I was unable to find any subsequent publications on comparative therapeutic efficacy or toxicity of (+)-, (–)-, and (±)-CP in humans. The second point deals with the stereochemistry of ifosfamide (IFF), which is a chiral constitutional isomer of CP wherein one of CP's CH_2CH_2Cl moieties is replaced with hydrogen and covalently bonded to the ring nitrogen. Racemic (±)-IFF received U.S. approval by the FDA in 1988 for use in combination with certain other approved antineoplastic agents for third-line chemotherapy of germ cell testicular cancer. Prior to this FDA approval of racemic IFF, Stec's laboratory had published the syntheses of enantiomerically pure IFF and two of its chiral metabolites. ^{31}P-NMR analysis of urine from patients treated with racemic IFF indicated "considerable stereoselectivity of *in vivo* formation of some chiral metabolites" of IFF.[50] Much later, in 1999, an extensive amount of experimental and clinical data for IFF and CP was reviewed by Williams and Wainer,[51] who stated that stereochemistry plays a minor role in the efficacy and toxicity of CP but is a major factor in neurotoxicity of IFF. Moreover, "[s]tudies have demonstrated that the use of a single IFF enantiomer, *R*p-IFF, would retain the unique antitumor efficacy of this agent, while eliminating the major source of the observed IFF-associated neurotoxin, *S*p-IFF."[51] To my knowledge, racemic IFF is still used in the clinic, and in my opinion there is insufficient published information to speculate about the contrasting roles of stereochemistry on the biological activities of CP and IFF.

CONCLUSIONS

The history of the elucidation of the chemistry which underlies the anticancer activity of CP provides a remarkable example of serendipity, and the unanticipated nature and complexity of the biochemical processes underlying the selective toxicity of CP toward cancer cells. The original 1950s drug-design premise based on elevated levels of putative phosphamidase activity in cancer cells that could selectively release toxic nitrogen mustard was proven to be incorrect. Nevertheless, CP was found to be a clinically useful anticancer drug that indeed required enzymatic activation, but that was found to occur in the liver, and involved oxidative hydroxylation of the C4 position in CP mediated by the cytochrome P450 system. Serendipitously, the resultant metabolite, 4-HO-CP, happens to have a chemically labile hemiaminal moiety that allows, under physiological conditions, rapid equilibration between *cis*- and *trans*-4-HO-CP, through AP, its ring-opened aldehyde-bearing tautomer. The serendipity continues in that the chemical constitution of AP happens to allow, under physiological conditions, fragmentation into acrolein and PM at a rate which is slow enough to allow circulation and cellular uptake of 4-HO-CP/AP, as PM exists

under physiological conditions as its negatively charged conjugate base (PM-), which is therefore not readily taken up by cells. And, by the same token, anionic PM- does not readily efflux from cells after its generation therein from AP. It is fair to say, I think, that an expert medicinal chemist could not reliably design, *a priori*, this overall multistep metabolic 'Trojan horse' (Figure 4) scheme for the ultimate release of PM/PM- from CP. The serendipity continues with the unforeseen intervention of not one, but two additional enzymatic conversions to produce the metabolites keto-CP and carboxy-CP, which are non-toxic and, serendipitously, occurs more rapidly in normal cells compared to tumor cells, thus giving rise to the selective toxicity of CP toward cancers. This additional pharmacology is also something that an expert medicinal chemist could not reliably design, *a priori*, in my opinion.

Immunosuppression is an aspect of CP chemotherapy that was not mentioned above, but is worth noting because it is yet another serendipitous aspect of CP. By the mid-1980s, and despite incomplete understanding of the exact mode of CP's immunosuppressive action, CP was being successfully used in certain nonmalignant diseases in which autoimmune phenomena are established or suspected in the pathogenesis of the disease.[52] Much more is now know mechanistically, and CP remains an important treatment for life-threatening autoimmune diseases where disease-modifying antirheumatic drugs have been ineffective. For example, systemic lupus erythematosus with severe lupus nephritis may respond to pulsed CP, and CP is also used to treat severe rheumatoid arthritis and multiple sclerosis.

All of these non-cancer beneficial uses for CP derived from chance, albeit to prepared minds, as in the well-known adage. In this regard, readers may be interested in the essay titled *On serendipity in science: discovery at the intersection of chance and wisdom* by Samantha Copeland.[53]

From the perspective of analytical tools, NMR spectroscopy proved to be a powerful addition to MS methods by providing real-time kinetics and compelling structural information. Currently, there are ~120 publications in PubMed that have CP and NMR/MRS in the title or abstract (and ~1,800 anywhere in the article). Aside from the mentioned ^{31}P-based *in vivo* imaging of CP in humans,[37] the treatment of human extremity sarcomas has been monitored by ^{31}P-MRS, and profiling of urinary acetate and citrate by ^{1}H-MRS following CP therapy.[54]

From a personal perspective, my initial working hypothesis (and that of Prof. Wojciech J. Stec) that the stereochemistry of CP would markedly influence enzyme-mediated metabolic pathways turned out not to be the case. However, the saying "never say never" applies to science, and it is possible that future stereochemical studies may provide new and possibly surprising information.

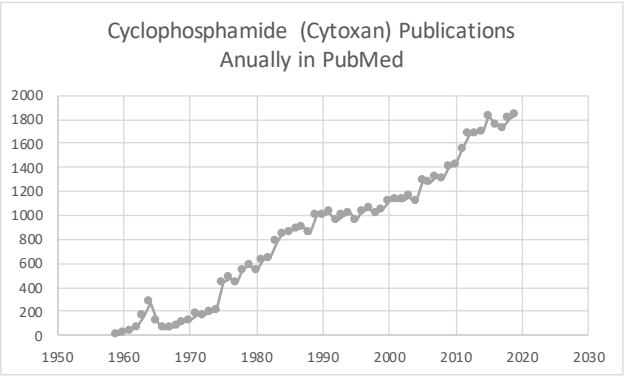


Figure 7. A chart of the number of annual publications in PubMed between 1959 and 2019 that have CP (or Cytoxan[55]) in the title and/or abstract. Between 1959 and 2019 there have been ~50,000 such publications, which show a continually increase trend, and over 1,800 in 2019.

After ~60 years of investigating CP, which has generated ~50,000 publications listed in PubMed that continue to show an upward trend (Figure 7), and ~4,000 structural congeners of PM listed in *Chemical Abstracts*, the amazingly serendipitous pharmacology of CP has been recently reported to have yet another surprise.

In 2019, Georg Voelcker, who has been investigating CP since the early 1970s, published a report[56] stating that "[a]ttempts to improve this drug found by a lucky coincidence have failed until now. The efforts failed because they were based on wrong assumptions about the mechanism of action of CP." Voelcker provides new data to support his proposal that 3-hydroxypropanal (HPA), $HOCH_2CH_2C(O)H$, "the overlooked CP metabolite" derived from AP by a phosphodiesterase, which he identified in 2017, has known[57] proapoptotic properties that contribute significantly to the anticancer activity of CP, in addition to the known[58] cytotoxic apoptosis induced by PM alkylation of DNA. If this new mechanism of action for CP involving HPA withstands further experimental testing, it would represent yet more serendipity in the amazingly serendipitous story of CP chemotherapy.

In closing, and in view of the historical chart of CP publications in Figure 7, it can be said with a high degree of certainty that in 2034, on the 75th anniversary of the approval of CP as a cancer drug, there will be far more publications than there are now. Based on extrapolation of a linear trend-line for the data in Figure 7 for 2019-2035, this future 15-year period would see ~30,000

additional CP-related publications. These future publications will likely include many investigations of the extent to which, and how, individual genomes influence CP activity and toxicity. This is already evident from a 2020 report noting that pharmacogenetic investigations have shown that CYP450 (which converts CP to 4-HO-CP), as well as aldehyde dehydrogenases (which converts 4-HO-CP to keto-CP), are associated with altered treatment response.[59] Since individual genetics similarly applies to glutathione-S-transferase isoenzymes, it was suggested that "[a] shift from genetic-based studies to whole-genome-based investigations of CP-associated markers may contribute to personalizing CP therapies.[59] Indeed, El-Serafi et al.[60] have clinically investigated and obtained data to support the potential importance of accounting for individual patient genotyping and levels of activating enzymes when personalizing treatment schedules in order to achieve optimal therapeutic drug plasma concentrations of CP.

ACKNOWLEDGEMENTS

The author is grateful for having had the opportunity to work with many coworkers and collaborators who contributed to, and coauthored, the CP references cited herein, and more on CP and its congeners that were not included. Many helpful comments and corrections provided by two anonymous reviewers are also appreciated. This historical account is dedicated to my long-time CP collaborator, and close friend, Prof. Wojciech J. Stec on the occasion of his 80th birthday, and my CP collaborator Dr. O. Michael Colvin (June 15, 1936 – March 16, 2013), who did pioneering work establishing the mechanism of action of CP and was one of the first investigators to use high-dose CP to treat solid tumors, now a common practice in bone marrow transplantation for breast and other cancers.

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Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Research Articles

Thermodynamics of Life

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OPEN ACCESS

Citation: Henry M. (2021) Thermodynamics of Life. *Substantia* 5(1) : 43-71. doi: 10.36253/Substantia-959

Received: Jun 02, 2020

Revised: Aug 25, 2020

Just Accepted Online: Aug 26, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Abstract. Biology is currently plagued by several fossil concepts that may be responsible for the current stagnation in medicine. Through a careful screening of the origins of thermodynamics, such fossils concepts have been identified: assumption that heat is a form of energy, assimilation of entropy to disorder, assimilation of death to states of maximum entropy, assimilation of ATP to the energy currency of living cells, non-recognition of entropy as a state function of the whole universe, belief that free energies are another kind of energy, self-referencing in the definition of life, ignorance of basic principles of quantum physics and more particularly of the importance of intrinsic spin, confusion between three different forms of reversibility, non-recognition that irreversibility is at the heart of living systems. After stowing of these concepts in the cabinet of useless and nasty notions, a fresh new look is proposed showing how life is deep-rooted trough the entropy concept in quantum physics on the one hand and in cosmology on the other hand. This suggests that life is not an emergent property of matter, but rather that it has always been a fundamental property of a universe filled with particles and fields. It is further proposed to dismiss the first (energy = heat + work) and third laws (entropy decreases to zero at zero Kelvin) of thermodynamics, retaining only the clear Boltzmann's definition of entropy in terms of multiplicity of microstates Ω, $S = k_B \times Ln\ \Omega$, and the second law in its most general form applicable to any kind of macrostates: $\Delta S_{univ} \geq 0$. On this ground, clear definitions are proposed for life/death, healthiness/illness and for thermodynamic coupling. The whole unfolding of life in the universe: Big Bang → Light → Hydrogen → Stars → Atoms → Water → Planets → Metabolism → Lipids → RNA's → Viruses → Ribosome → Proteins → Bacteria → Eukaryote → Sex → Plants → Animals → Humans → Computers → Internet, may then be interpreted as a simple consequence of a single principle: $\Delta S_{univ} \geq 0$. We thus strongly urge biologists and physicians to change and adapt their ideas and vocabulary to the proposed reformulation for a better understanding of what is life and as a consequence for better health for living beings.

Keywords: entropy, life, death, thermodynamics, irreversibility, heat, time.

INTRODUCTION

Some time ago, it has been advocated that scientific knowledge has generated during its rapid expansion a certain number of conceptual fossils.[1] Among the identified fossils we have: Newton's three laws, actions at distance in physics, existence of several forms of energy, space 'full of nothing' but having properties, hysteresis curves in ferromagnetism and entropy as a

measure of disorder. It is worth noting that such fossils exist because they are vestiges of ways of thinking that are no more adapted to modern scientific knowledge. The trouble is that fossils are still well alive in the world of scientific teaching and that they are the first creatures met by young students learning mechanics, electromagnetism, thermodynamics, chemistry and biology. Being sprinkled by the dust accumulated over eons, fossils are still haunting nostalgic scientific minds writing publications or books. The field that is the most plagued with fossil thinking is obviously biology and by extension medicine. Conversely, the field that is the less contaminated by fossils is physics owing to the occurrence of two great revolutions: general relativity and quantum mechanics. Fossils also spontaneously contaminate thermodynamics and chemistry, but as soon as such scientists become acquainted with quantum physics, the contamination disappears quickly. The trouble is that biologists and physicians are hardly trained in quantum physics and have thus a minimal chance of stowing their fossils in the cabinet of useless concepts.

This is very unfortunate, as biology and medicine have to deal with the life phenomenon, a hassle not encountered in chemistry or physics. However, it appears that thermodynamics is a way of thinking that is shared by physics and chemistry on the one hand and by biology and medicine on the other hand. So, there is good chance that by focusing on thermodynamics, biologists and physicians may be able to make their revolution to cast a firm and non-fossil bridge over the chasm separating inert from living matter (see figure 1 in reference [2]). In the following, we will address the problem starting from first principles with the aim of having a clear picture on how life has appeared on earth without any violation of the second law. It is our feeling that some conceptual fossils that ought to be exorcized currently hinder useful progresses in biology and medicine. By stowing these fossils at their right place, one may hope initiating the same kind of revolution that has affected chemistry and physics at the dawn of the twentieth century. The basic aim here is not introducing totally new yet unknown concepts, but rather reinterpreting ancient ones at the light of quantum theory and at the scale of the whole universe. In the new proposed paradigm, life should no more be perceived as a highly improbable event, but rather as an inexorable consequence of universe's birth some 14 billion years ago.

LIFE AND DEATH

One of the biggest fossils that plagues thermodynamics is the assimilation of entropy with disorder. Every scientist, even the most brilliant ones, may be tempted to use such a misconception either in teaching or in research. In fact, the misconception arises as soon as Boltzmann's relationship $S = k_B \cdot \mathrm{Ln}\ \Omega$ is not recognized as one of the most fundamental principle ruling universe's evolution starting from inert matter and ending up in living matter and consciousness. Having an unclear idea of what is lurking behind the Greek letter Ω is the main hurdle that prevents a good understanding of what is entropy. Being ignorant of the real nature entropy triggers a quasi-automatic switch of attention towards a closely related concept: energy E.

A good starting point is to spend some time around a crucial question: What is life? And one of the most obvious answers was provided by the Greek philosopher Aristotle some 2 500 years ago by noticing that spontaneous motion was an essential attribute of any living thing. And as soon as motion is identified with life, it follows the following logical inference: "*Quo cicior motus, eo magis motus*", stating that the faster a motion, the more of a motion it is. This innate property of motion then enters in deep resonance with the fact that the more life does, the more life it is.[3] Then enters the great Sir Isaac Newton showing that motion may be changed by applying forces (*vis impressa*) that could be viewed either as a temporal gradient, $f = dp/dt$, of the amount of motion $p = m \cdot v$ (where v is the velocity of a given mass m) or as a spatial gradient, $f = -dE/dr$, of a potential energy E. Later, correcting Descartes's misconception of the amount of motion, Gottfried Leibniz introduced its *vis viva* meaning "living force" that was not a force at all, but rather kinetic energy ($E = p^2/2m$). With the hope of divorcing from Aristotle's duality between actuality (observed motion) and potentiality (virtual motion), it was finally decided to consider a single unifying theoretical concept (energy) that have a single manifestation in time (kinetic energy related to mass) and many manifestations in space (potential energies related to abstract fields derived from the presence of masses or electrical charges).

Consequently, with energy responsible for motion and with motion being an obvious attribute of life, an obvious connection between energy and life could be established and is still perpetuated in modern biological thinking where every event is analyzed in terms of available energy supposed to be stored in the "high-energy" part of a molecule named adenosine triphosphate (ATP). The thesis defended here, is that such a view is just a highly fossilized dogma preventing us to really understand what is life and one of its most deadly manifestation: cancer. The trouble with such a dogma is that quite great minds have been obliged to be engaged

into incredible intellectual contortions to explain what is life. A most prominent contribution was obviously Erwin Schrödinger's introduction in biological thinking of a totally new crazy concept nicknamed negentropy.[4] Schrödinger's reasoning is that entropy being disorder, a living organism, an obviously ordered thing, avoid decay by eating, drinking and breathing, that is to say through the existence of a metabolism. Being an expert in physics he knew perfectly well that any calorie is worth as much as any other calorie and that the overall energy content of an organism is stationary as well as its material content. Here an exact quote on how he was finally led to introduce this new concept:

> *Everything that is going on in Nature means that an increase in the entropy of* ***the part of the world*** *where it is going on. Thus, a living organism continually increases its entropy and thus tends to approach the dangerous* ***state of maximum entropy, which is death****. It can only keep aloof from it, i.e. alive, by continually drawing from its environment negative entropy — which is something very positive, as we shall immediately see. What an organism feed upon is negative entropy.*

I have put under bold character the slippery parts of the argument. The first misconception is that the second law of thermodynamics stating that entropy is doomed to always increase in time does not concern a part of the world but the *universe taken as a whole*. The second misconception is to associate death to a state of maximum entropy. This is just utterly wrong as assuming that life is motion means that a state of maximum entropy is also a state of maximum in motion. To keep coherence with associating motion and life, one should state that death, *i.e.* absence of motion, should be better associated with the crystalline state observed close to a temperature of 0K and corresponding to a state of null entropy (Nernst's theorem). Accordingly, every physician knows that just after death, the body undergoes a transition from a gel state of high entropy towards a fully rigid state named *rigor mortis* of lower entropy.

Subsequent decomposition corresponding to an increase in entropy with liquefaction and gases escapes should be attributed to an intense activity from microorganisms that use the dead corpse as a source of food. One may also, by using suitable chemical compounds, inhibit such a microbial activity. If this is the case, the dead body increases its rigidity until achievement of the mummy state where crystallinity becomes so high and entropy so low that the dead body can remain unaltered with full exquisite structural details during a thousand of years for humans and during millions of years for animal fossils.

Obviously, Schrödinger being an expert in theoretical physics with absolutely no experience in medicine cannot be blamed for the second mistake made by trying associating death with states of maximum entropy. The need for a sign reversal in entropy is in fact a logical conclusion of such a wrong initial assumption. But, despite distilling fundamentally wrong ideas in biology, Schrödinger's little book has been greatly influential in inspiring a number of pioneers of molecular biology taking for granted that the origin of life is the same thing as the origin of replication. However, for scientists thinking that metabolism was more central to life than replication, Schrödinger's book was just a sword cutting through water. Quoting for instance Linus Pauling, Nobel Prize in Chemistry (1954):

> *When I first read this book, over 40 years ago, I was disappointed. It was, and still is, my opinion that Schrödinger made no contribution to our understanding of life.*[5]

Concerning Max Ferdinand Perutz, Nobel Prize in Chemistry (1962):

> *Sadly, however, a close study of his book and of the related literature has shown me that what was true in his book was not original, and most of what was original was known not to be true even when the book was written (…). The apparent contradictions between life and the statistical laws of physics can be resolved by invoking a science largely ignored by Schrödinger. That science is chemistry.*[6]

Finally, for the theoretical physicist Freeman H. Dyson, Henri Poincaré Prize (2012):

> *Schrödinger's account of existing knowledge is borrowed from his friend Max Delbruck, and his conjectured answers to the questions that he raised were indeed mostly wrong. Schrödinger was woefully fully ignorant of chemistry, and in his isolated situation in Ireland he knew little about the new world of bacteriophage genetics that Delbruck had explored after emigrating to the United States.*[7]

In fact, Schrödinger's view was more oriented towards viruses that are just replicating molecules rather than towards living cells that could reproduce owing to the existence of a metabolism.

Alas, Schrödinger was recipient of the Matteucci Medal (1927), the Nobel Prize in Physics (1933) and the Max Planck Medal (1937). At this level of honors, everything you say is taken as golden words, even when these words have been expressed in a domain very far from your field of expertise. A striking example of the paralyzing effect of Schrödinger's two mistakes is provided by this passage of Szent-Györgyi's little book on water and cancer (chapter IV, p. 40).[3]

The more life does, the more life it is; the more negative entropy is liberated, the more can be retained of it. Life supports life, ***function build structures, and structure produces function****. Once the function ceases, the structure collapses, it maintains itself by working. A good working order is thus the more stable state. The better the working order, the greater its stability and probability. In inanimate systems the most stable state is at the minimum of free energy and maximum of entropy. This is 'physical stability'. In living systems the opposite is true.* ***The greatest stability is at the maximum of free energy*** *and* ***minimum of entropy****, which corresponds to the* ***best working order****. This is 'biological stability'.*

Again, the man writing these words was recipient of the Nobel Prize in Physiology and Medicine (1937) and of the Albert-Lasker Prize (1954).

The first statement underlined with bold characters is a pretty good example of a circular argument, that is, an argument that assumes the conclusion as one of its premises. Such statements should be systematically avoided, owing to their inevitable evolution towards vicious circles, chains of events in which the response to one difficulty creates a new problem that aggravates the original difficulty. The difficulty of making progresses in medicine nowadays may be directly related to this first circular argument where life is defined as being life. The last statement is just the consequence of Schrödinger's initial mistake. Here we are now facing a wrong argument, as anybody well-trained in thermodynamics knows that a state of maximum free energy is always unstable, *i.e.* never stable. Concerning the last sentence, again it is worth stressing that in thermodynamics, the state of a minimum of entropy is the crystalline state, a state where no kinetic energy is available to perform work. If undisturbed, a crystal will always remain a crystal for eternity with absolutely no tendency to perform any kind of work, as it corresponds to a state of maximum potential energy. Here we are facing the reverse situation where an expert in biology with very little training in physics uses its scientific authority for talking outside its expertise domain. The pity was that Szent-Györgyi was on the good track by associating water and metabolism, but that he was also paralyzed by Schrödinger's wrong ideas about entropy.

ORIGIN OF LIFE

A first obvious point is the failure of modern biology to clearly explain how life has appeared on earth. Nowadays, it is obvious that acetyl-coenzyme A deriving from pyruvate decarboxylation is the universal food of any kind of living cell. However, such a statement may be wrong as it has been demonstrated that pyruvate may be engaged in a purely abiotic cycle where citrate is replaced by 4-hydroxy-2-keto-glutarate (HKG).[8] As this HKG-based cycle is able running without the help of enzymes and consuming pyruvate, glyoxylate and hydrogen peroxide H_2O_2 instead of dioxygen O_2, it is a good candidate for a very primitive way of unrestricted proliferation.

Yet another tacit major assumption of biology is that adenosine triphosphate (ATP) should be the universal energy carrier of any living entity. However, it has been recently demonstrated that ATP has properties of a biological hydrotrope through its ability to solubilize hydrophobic molecules in aqueous solutions.[9] Its main role would thus be to prevent the formation harmful protein aggregates as well as a being a powerful remover at millimolar scale of previously formed aggregates.

It has long being pointed out by Nobel's price winner Albert Szent-Györgyi that water should be considered as the web of life and that bioenergetics is but a special aspect of water chemistry:[3]

Biological oxidation is, as rule, not a coupling with O_2, but simply a replacement of the H's by the water, H and OH, which makes the substance gradually richer in O till eventually only CO_2 and H_2O remain. Oxygen comes only as a final electron acceptor. All this may be common knowledge. I mention it because we tend to concentrate only on the substances to be split, joined, or oxidized and forget the molecule which plays the central role in all these processes, water.

Moreover, in a quite remarkable insight, Szent-Györgyi could foresee that during anaerobic life, a pool of H's have been constantly on tap with sufficient food to fill the pool with almost no limit to proliferation. When O_2 appeared as a waste of photosynthetic activity, it was possible to turn off the tap of the H-pool during the so-called great oxidation event (GOE), opening the way to differentiation and thus to the building of complex multi-cellular organisms. However, when the cell divides, it has to break down its bulky oxidative mechanism and revert to the more archaic use of the H-pool.

The best way to get a reasonable scenario for life apparition on earth is here to trust mathematicians and not biologists. Accordingly, biologists are concerned with nowadays life and following Schrödinger's book have taken for granted that the duplicative aspect of life is primary and the metabolic aspect secondary. Such a polarization towards the idea that metabolism is governed by gene expression being obvious for a modern cell, the good question is to wonder if the reverse order (*i.e.* metabolism controlling gene expression) was not the rule in the past[7]. As there is a fierce debate in biology

about what was the good order at the very beginning, the best way is to get a clue from mathematics. This is because the very notion of time is meaningless in mathematics with no dependency on precise material configurations contrary to living cells that are made of matter and subjected to the arrow of time. Moreover, mathematicians have created computers that are not precisely alive, but nevertheless share with living cells the ability to deal with information.

It is a well-known fact that automatons have been invented and developed by John von Neumann; the man who gave to quantum physics its mathematical foundations. For developing computers, von Neumann has understood that any automaton should have two essential components. A first one, is hardware for processing information, the second one being software for embodying information into instructions. Transposed to a living cell, von Neumann's mandatory dualism points to proteins (metabolism) as hardware and nucleic acids (replication) as software. Could we now imagine what would be the behavior of hardware without software? Such a situation is encountered as soon as the computer enters into an endless loop. Such an automaton is doomed to crunch numbers independently for as long as it is alimented. For bacteria, this is unlimited growth while for multicellular organisms we have cancer. Now let's reverse the problem by asking what would be the behavior of software without hardware? Here again, we have an answer for both automatons and living cells: viruses. The fact that the same term has been here chosen for a stuff made of inert matter (computer) as well as for a living stuff (cell) comes from the fact that the material configuration embodying information does not matter. Of course viruses are obligatory parasites that needs a cooperative host equipped with hardware for being able to undergo replication. And from such a viewpoint a clear order emerges: metabolism first, replication second. As such a conclusion is suggested from the study of computers, it should be seriously considered as a fundamental truth for all systems implicated into information processing. The whole scenario for life apparition on Earth is now clarified and may be summarized by a series of successive events, each one requiring presence of its predecessor to be able generating its successor:

Big Bang → Light → Hydrogen → Stars → Atoms → Water → Planets → Metabolism → Lipids → RNAs → Viruses → Ribosome → Proteins → Bacteria → Eukaryote → Sex → Plants → Animals → Humans → Computers → Internet → ?

The first events from Big Bang to Planets are taken from physics (cosmology and quantum mechanics) and will not be discussed in details here. Please however note that according to this fundamental life-development scenario that hydrogen should not be considered as an atom, but rather as a combination of two elementary particles (proton and electron) generated by the Big Bang that have generated quarks for building nucleons and leptons for building atoms after association with nucleons. This separation is important for stressing that hydrogen should be considered as a universal "fuel" in our universe, not only for stars (proton eaters), but also for living cells (proton plus electron eaters). Following nucleosynthesis in stars leading to supernova explosion, synthesized atomic nuclei were dispersed within the universe to form atoms and molecules on cool bodies. Among all the possible atomic combinations, we have chosen to highlight water H_2O, as this substance has always been associated with occurrence of life. From a purely statistical viewpoint, there is in fact no other possible choice as ordering chemical elements by decreasing cosmic abundances, we get the following order: H, He, O, Ne, N, C, Si, Mg, Fe, S, Ar, Al, Ca, Na, Ni, P, Cl, K.[10] Ignoring helium (He), a closed shell unreactive atom, the most abundant nuclei prone to accept protons and electrons to form a neutral combination is oxygen. Consequently, if we admit that life is a fundamental attribute of the universe, it logically follows that its material expression as a movement should involve hydrogen, oxygen and their low-temperature marriage: water. Then, to control these natural moves, life also needs structures and from the cosmic abundance sequence, the next three recruited nuclei should be nitrogen, carbon, and sulfur as neon is, like helium, a closed shell unreactive atom. Consequently, the following gases should, for purely statistical reasons, be important for life manifestation: water = (H_2, H_2O, O_2, O_3) and structure = (NH_3, CH_4, C_2H_2, C_2H_4, N_2, NO, CO, CO_2, HCN, H_2CO, NCO, HNCO, H_2S, COS).

Besides these gaseous combinations, oxygen the most abundant element after hydrogen would also combine with silicon, sodium, potassium, magnesium, calcium, aluminum and carbon leading to important crust minerals such silico-aluminates (Na, K, Ca, Mg, Si, Al, O), dolomite (Ca, Mg, C, O), apatite (Ca, P, O) and pyrite (FeS_2) together with sodium chloride (NaCl) in oceans. For the mantle, we should have obvious (Mg, Fe, Si, O) combinations in contact with a (Ni, Fe) metallic core at the very center. Let us now check that gases (C_2H_2, CO, CO_2, HCN, H_2CO, HNCO, COS) could be used form creating software (information embodying, replication). Assuming a metabolism provided by cosmic rays, what kind of software we may expect? Here is a possible list by considering addition reactions assisted by cosmic radiations (symbol γ):

Carbohydrates (ribose if n = 5): n H_2CO + γ = $HOCH_2$-$[CH(OH)]_{n-2}$-CHO
Adenine (A): 5 HCN + γ = $C_5H_5N_5$
Guanine (G): 4 HCN + HCNO + γ = $C_5H_5N_5O$
Uracil (U): C_2H_2 + 2 HCNO + γ = $C_4H_4N_2O_2$
Cytosine (C): C_2H_2 + 2 HCNO + HCN + γ = $C_4H_5N_3O$ + CO
Thymine (T): C_2H_2 + HCNO + HCN + H_2CO + γ = $C_5H_6N_2O_2$

Accordingly, nitrogen heterocycles are commonly found in carbonaceous chondrites that are highly porous meteorites rich in carbon and water.[11] After Earth accretion and following the great deluge that have filled the oceans, one may also consider alteration of apatite $Ca_5[PO_4]_3(OH)$ by water and carbon dioxide assisted by the intense ultraviolet radiation in provenance from the Sun:

$$Ca_5[PO_4]_3(OH) + 3\ [HCO_3]^{\ominus} + H_2O + 2\ CO_2 + \gamma = 3\ [PO_2(OH)_2]^{\ominus} + 5\ CaCO_3$$

The basic building blocks of ribonucleotides [1'(A,G,C,U]-Ribose-(5')CH_2-O-PO_2-(OH)]$^{\ominus}$ may then further be assembled into RNA's, with the help some H-pool and most probably clays (silico-aluminates). Obviously, one may also use the intense energy provided by cosmic rays to create 20 building blocks for an organic hardware at the surface of meteoric materials for instance (Table 1):

Reduced amino acids:
{(n–p) CO + CO_2 + p COS + q HCN} + m H_2 + γ = $C_{n+q+1}H_{2(m-n)+q}N_qO_2S_p$ + n H_2O

Oxidized amino acids:
{n CO + 2 CO_2 + q HCN} + m H_2 + γ = $C_{n+q+2}H_{2(m-n-k)+q}N_qO_{4-k}$ + (n+k) H_2O

For the existence of left-handed amino acids and the virtual exclusion of their right-handed forms, one may invoke the asymmetric distribution of neutrinos emitted by a supernova[12]. Further condensation to form polypeptides has probably occurred within the van der Waals gap of clays minerals thanks to carbonyl sulfide for instance[13]. Clays or iron sulfur bubbles (see[7] for details concerning plausible scenarios and references) would be necessary for protection of these fragile polymers from intense ultraviolet radiations emitted by the Sun. Obviously, lacking nitrogen-containing gases, one may also envision synthesis of fatty acids at the mouth of black smokers for instance where the reducing power of the magma meets water (see[8] for a more detailed story):

Table 1. Encoding of the 20 standard amino acids according to the number of carbon monoxide (n), carbonyl sulfide (p), cyanydric acid (q), dihydrogen (m) and reduction level (k) needed for their synthesis at the surface of meteorites thanks to cosmic radiations.

n	p	q	M	Symbol	Letter	k	Formula
0	0	1	2	Gly	G	-	$C_2H_5NO_2$
0	0	1	4	Ser	S	1	$C_3H_7NO_3$
0	0	2	4	Asn	N	1	$C_4H_8N_2O_3$
0	0	4	7	Arg	R	2	$C_6H_{14}N_4O_2$
1	0	1	4	Ala	A	-	$C_3H_7NO_2$
1	1	1	4	Cys	C	-	$C_3H_7NO_2S$
1	0	1	4	Asp	D	0	$C_4H_7N_2O_3$
1	0	1	6	Thr	T	1	$C_4H_9NO_3$
1	0	2	6	Gln	Q	1	$C_5H_{10}N_2O_3$
2	0	1	6	Glu	E	0	$C_5H_9NO_4$
2	0	3	5	His	H	-	$C_6H_9N_4O_2$
3	0	1	8	Val	V	-	$C_5H_{11}NO_2$
3	0	1	7	Pro	P	-	$C_5H_9NO_2$
3	1	1	8	Met	M	-	$C_5H_{11}NO_2S$
3	0	2	9	Lys	K	-	$C_6H_{14}N_2O_2$
4	0	1	10	Leu	L	-	$C_6H_{13}NO_2$
4	0	1	10	Ile	I	-	$C_6H_{13}NO_2$
6	0	1	12	Tyr	Y	1	$C_9H_{11}NO_3$
7	0	1	12	Phe	F	-	$C_9H_{11}NO_2$
8	0	2	13	Trp	W	-	$C_{11}H_{12}N_2O_2$

{(n-1) CO + CO_2} + (2n H_2 + magma) = $C_nH_{2n+2}O_2$ + (n-1) H_2O

Such fatty acids would allow formation of oily little bags holding inside their cavity a more or less random collection of organic molecules. Such proto-cells would concentrate organic matter and after becoming too big would be cut in half producing two daughters inheriting in a statistical way the chemical machinery.

At this stage, the oily bags would be confronted to the problem of keeping a good solubility for their large amount of watery organic matter. A crucial step would thus be selection of ATP as a powerful hydrotrope.[9] This is because ATP becomes essentially a ribonucleotide after removal of two phosphate groups. So, if RNA's could be formed from AMP within these oily bags, the creation of ATP under low water activity conditions is not unlikely. But RNA is a molecule able to replicate itself that could be transferred from bag to bag carrying, at each transfer, deterministic genetic information instead of the statistical whole chemical machinery.

HEAT, METABOLISM AND ENERGY

Having a clear scenario of life apparition on Earth, the only remaining obscure point that remains to be clarified is the physical nature of a primitive metabolism. During the eighteenth century, the nature of heat was a deep question related to the question of how improving steam engines to get the maximum efficiency from a given amount of combustible. A decisive step was made in 1784 by the French chemists Antoine Laurent de Lavoisier and Pierre Simon de Laplace after invention of an ingenious ice-calorimeter measuring the amount of heat emitted during combustion and respiration. By measuring the oxygen consumed during respiration it was thus proven that combustion and respiration were one and the same and that the amount changes depending on human activities: exercise, eating, fasting, and sitting in a warm or cold room[14]. However, Benjamin Thomson, Count Rumford, in a famous experiment made in 1798 showed that the heat generated in the process of boring cannon was a definite, measurable quantity, which did not reduce as long as the experiment was continued. It thus follows that the source of the heat generated by friction in these experiments, appeared evidently to be inexhaustible.[15] For Rumford, it was obvious that the only thing that could be produced without any limit from mechanical work was *motion*, meaning that heat should indeed be a form of motion.

But at that time heat was not perceived as motion but rather as a kind of immaterial fluid, named *caloric*, that could be exchanged between material bodies depending on their thermal state measured by their respective temperatures. In 1824, it was even possible to forge a physical unit, *the calorie*, as being the amount of heat necessary to change the temperature of 1 gram of water from 14.5 to 15.5 °C under atmospheric pressure. The same year, the French engineer, Sadi Carnot makes a decisive contribution with the happy idea of a reversible engine that would be able to turn the shaft backwards, delivering the same work w back to the engine and the same heat q back to the high-temperature reservoir[16]. He was then the first perceiving that no heat engine could be more efficient than a reversible engine operating between two temperatures t_2(reservoir) < t_1(heat's source). Accordingly, if Carnot's principle were wrong, then it would be possible to build machines that would run forever, delivering an infinite amount of work without any expenditure of fuel (perpetual motion machines of the second kind).

One of the big advantages of reversible heat engines is that they are universal devices, working independently of the working substance (not necessarily steam) or on the mode of operation (internal machinery does not matter). However, Carnot could not give a quantitative criterion for reversibility, meaning that his decisive contribution was in fact completely ignored. In 1840, Dr. Julius Robert von Mayer, a German physician, while surgeon to a Dutch India vessel cruising in the tropics, observed that the venous blood of sailors seemed redder than venous blood usually observed in temperate climates.[17] Mayer then reached the conclusion that the cause must be the lesser amount of oxidation required to keep up the body temperature in the tropics, suggesting that the body was a thermal machine dependent on outside forces for its capacity to act. Such a revolutionary idea was however completely ignored by physicists until 1847, when another German physician, Hermann von Helmholtz, had been independently led to the idea of energy conservation. Meantime in England, James Prescott Joule was going on from one experimental demonstration to another, suggesting the existence of a universal mechanical equivalent of heat. In 1845, after several years of hard experimentation in his kitchen, Joule was finally supported by William Thomson, (later Lord Kelvin), for a definitive establishment of the law of conservation of energy.

It was only after recognition of a mechanical equivalent of heat by Joule and Kelvin that reversible efficiency e_r was established to be a universal function of the temperatures.[18] Introducing its universal temperature scale that is independent of the properties of any particular substance, Kelvin could show in 1854 that the efficiency e of real heat engine efficiency should obey the following inequality:

$$e = 1 - \frac{q'_2}{q_1} \leq e_r = 1 - \frac{T_2(hot)}{T_1(cold)} \Leftrightarrow \frac{q_1}{T_1} + \frac{q_2}{T_2} \leq 0$$

Here e_r is Carnot's universal reversible efficiency, q_1 being the heat received by the cold reservoir and $q'_2 = -q_2$, the heat discharged from the hot source, with equality if and only if the engine is reversible.

ENERGY AND SPIN

At this stage (1854), we meet another fossil concept stating that heat should be a form of energy. The wrongness of such an idea may be easily demonstrated by the fact that heat can be created at will from friction, whereas mechanical energy cannot be created or destroyed. It follows that enunciating the first law of thermodynamics as $E_{int} = q + w$, where E_{int} is a total internal energy, q heat and w mechanical work is evil science.[19] Adding

two quantities measured with the same physical unit (joule) but of different nature explains why thermodynamic structure appears strange and confusing relative to other fields of physics, where such an error is never made. It is thus time to dive into quantum theory, a science where, contrary to thermodynamics, energy has a clear definition, as being the eigenvalue of an *ab initio* Hamiltonian operator acting on a Hilbert's space spanned by the eigenvectors of the Hamiltonian operator (Heisenberg's representation). Accordingly, at this level of theory to each system composed of N positively charged nuclei associated to N negatively charged electrons corresponds a characteristic discrete energy spectrum $\{\varepsilon_n\}$ indexed by an integer n called a quantum number. And here a very strange thing occurs, as instead of putting the N electrons into the ground state ε_1 of the lowest energy in order retrieving the lowest possible energy, electrons occupies not only the ground state levels but also other higher energy levels up to a maximum value (n_{max}). The rule governing the filling of these high energy levels follows from a property called "spin" taking the value one-half for protons, neutrons or electrons.

Accordingly, as electrons are not classical particles, but rather quantum entities ruled by a wave-function, they should obey Pauli's exclusion principle stating that a non-degenerate energy level ε_n cannot hold more than 2 electrons: one spin 'up' (eigenvalue +1/2) and the other one spin 'down' (eigenvalue -1/2). For highly symmetric molecules, it may happen that two or more energy levels could be degenerated, that is to say that a number m of quantum states share the same eigenvalue. In such a case, Hund's rule states that the configuration displaying the lowest energy, called the "ground state", is the one having the maximum intrinsic spin as well as the maximum angular momentum. The energy spectrum $\{\varepsilon_n\}$ associated to any combination of nuclei and electrons is nowadays readily obtained from scratch by solving Schrödinger's equation under a various set of approximations. Thus, filling each energy level with ν_n electrons (ν_n = 2, 1 or 0) starting from the most negative energy value, the total molecular energy when all nuclei are at their equilibrium positions may be written:[20]

$$E_{molec} \approx 1.55 \sum_n \nu_n \varepsilon_n$$

For a stable molecule, all filled level (ν_n = 2) should be of low energy ($\varepsilon_n < 0$), while all empty levels (ν_n = 0) should be of high energy ($\varepsilon_n > 0$), meaning that E_{molec} becomes more and more negative as the total number of electrons increases. When $\varepsilon_n < 0$ (bonding state), there is a good screening by the negatively charged electrons of the highly repulsive nuclei-nuclei interaction. In such a bonding state nuclei are engaged in a *chemical bond* with a bond order of 1. Conversely, when $\varepsilon_n > 0$ (anti-bonding state), there is bad screening of the positively charged nuclei by the electrons, leading to their separation and consequently the bond order is counted as -1. By summing all bond orders over all occupied states, a total bond order is obtained that is usually 1 (single bond), 2 (double bond) or 3 (triple bond). If the bond order is zero, it is impossible to make chemical bonds, a situation encountered with neutral inert gases such as helium, neon and argon that exist only under a mono atomic state. Moreover, as electrons repel each other's, removing one electron to form a cation has a stabilizing effect on the energy levels whose energies become more negative. Similarly, adding an electron to form an anion has an overall destabilizing effect on the energy levels whose energies become less negative.

Having an energy levels diagram in hand and electrons obeying Pauli's exclusion principle, two essential energy levels ruling chemical reactivity should be considered (called *frontiers orbitals*). These two levels are the HOMO (acronym for *highest occupied molecular orbital*) that fixes the spin state and the LUMO (acronym for *lowest unoccupied molecular orbital*), the first empty level located just above the HOMO. Now, a first general rule states that the larger the HOMO-LUMO gap, the higher the chemical stability. This rule has for immediate consequence that the lower the HOMO-LUMO gap, the more reactive and unstable the species is. These rules explain why a radical having only a SOMO that has both HOMO and LUMO character, *i.e.* a zero HOMO-LUMO gap, belongs to the class of the most unstable and reactive species. And as radicals can be very dangerous species for other non-radical molecules, their role in a living cell is always twofold depending on concentration. At low concentration and high water activity, radicals act as redox signaling messengers with important regulatory functions leading to the so-called positive physiological stress or eustress.[21] At high concentration and low water activity, the same radicals may be responsible for deleterious effects on DNA, polyunsatured fatty acids (PUFAs) and proteins leading to the so-called negative physiological stress or distress. Such a stress-response hormesis is now well documented, meaning that radical scavengers may act either as protective agents or as poisons and should be used with extreme care. Moreover, as terms such as ROS, RNS and antioxidants are quite vague, it is very difficult to forecast what will be the effects of redox-active species.

It is also the HOMO-LUMO frontier orbitals that allow deciding if a molecule should be considered as

an acid oxidant or as a base reductant. Accordingly, to behave as an acid or oxidant, a molecule should be able to *accept electrons* and needs for that to have a LUMO of negative energy. Reciprocally, to behave as a base or reductant, a molecule should be able to *give electrons* and thus needs to have a HOMO of positive energy. Within such a frame any chemical transformation means involvement of a HOMO on one reactant (the base or the reductant) interacting with a LUMO on another reactant (the acid or the oxidant). Depending on the relative energy order of these frontiers orbitals, all chemical reactions may be grouped in just two classes:

i) Acid-base reactions when the HOMO of the base has a lower energy than the LUMO of the acid. Such reactions are easily recognized as in such cases oxidation numbers of all atoms remains the same before and after the reaction. In aqueous solutions acid-base interactions usually involves transfer of a proton $H^{\oplus}$.

ii) Redox reactions when the LUMO of the oxidant has a lower energy than the HOMO of the reductant. In such a case some oxidation numbers are doomed to change before and after the reaction through exchanges of one or two electrons.

It is also worth noticing that according to Noether's theorem, the covariance of the equations of motion regarding a continuous transformation with n parameters implies the existence of n quantities, or constants of motion, i.e., conservation laws.[22] More precisely, for each infinitesimal generator of a given continuous Lie group associated to a variable r, it exists a momentum p that remains constant in time and a relativity principle for the variable r. For instance, physical laws of mechanics and electromagnetism are known to be covariant under Poincaré's symmetry group ISO(3,1) having 10 infinitesimal generators. Then, for any infinitesimal translation in time (r = t), the associated conserved momentum is energy (p = E) with arbitrariness in the origin of time. Likewise, for any infinitesimal translation in space (r = x, y, or z), linear momenta (p = $m{\cdot}v_x$, $m{\cdot}v_y$ and $m{\cdot}v_z$) are conserved with arbitrariness in the origin of space. Moreover, for any infinitesimal boost in speed of the center of mass (r = v_x^{CM}, v_y^{CM} or v_z^{CM}), the coordinates of the center of mass at t = 0 (p = $x_{CM}°$, $y_{CM}°$ and $z_{CM}°$) are conserved with arbitrariness in the absolute speed of center of mass. Finally, for any infinitesimal rotation in space (Euler's angles r = α, β, γ), there is conservation of angular momenta (p = L_α, L_β and L_γ) with arbitrariness in the orientation of space. Consequently, at the mechanical level, although the coordinates and velocities of the constituent parts of an isolated mechanical system may change with time, the sum of all the kinetic and potential energies of all the constituent parts (total energy) is a constant of the motion and has a fixed value, E (Noether's theorem).

Table 2. The Wigner-Witmer spin correlation rules. 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: $|S_A + S_B|$, $|S_A + S_B - 1|$, $|S_A + S_B - 2|$,…, $|S_A - S_B|$.

Reactant A	Reactant B	Total allowed spin
Singlet (S = 0)	Singlet (S = 0)	Singlet (S = 0)
Doublet (S = ½)	Doublet (S = -½)	Singlet (S = ½ - ½ = 0)
Triplet (S = 1)	Triplet (S = -1)	Singlet (S = 1 – 1 = 0
Singlet (S = 0)	Doublet (S = ½)	Doublet (S = 0 + ½ = ½)
Triplet (S = 1)	Doublet (S = -½)	Doublet (S = 1 - ½ = ½)
Singlet (S = 0)	Triplet (S = 1)	Triplet (S = 1)
Doublet (S = -½)	Quartet (S = 3/2)	Triplet (S = 3/2 - ½ = 1)
Doublet (S = -½)	Quintet (S = 2)	Quartet (S = 2 - ½ = 3/2)

Another point following from Noether's theorem is that spin is basically an intrinsic angular momentum that should, as mechanical energy, never change even if molecules are engaged in chemical transformations. This second conservation properties gives rise to the so-called Wigner-Witmer correlation rules that determine the tendency of a reacting system to conserve spin angular momentum.[23] These Wigner-Witmer correlation rules (see Table 2) are of the utmost importance because if they are not satisfied for a given reaction, the reaction will occur, in case of small spin-orbit coupling, only at a very slow rate without a catalyst. This is why you may perfectly mix hydrogen and oxygen in stoichiometric proportions without any violent reaction, even though hydrogen is a one of the strongest reductants and oxygen one of the best oxidants, just after fluorine. This potentially highly exothermic reaction cannot occur in without sparkles, heat or light, simply because it is spin-forbidden (see below). It is the HOMO frontier orbital that allows predicting what will be the spin of a molecule, with three main possibilities.

i) The number of electrons is even and the HOMO is not degenerated. In such a case, the total spin of the molecule is zero corresponding to a *singlet* spectroscopic state (S = 0). The water molecule is a good example of such a possibility. In fact, most stable molecules fall in this first category.

ii) The number of electrons is odd and the HOMO is again not degenerated. In such a case, the species is called a *radical* having a total spin of one half corresponding to a doublet spectroscopic state. In such a case the HOMO becomes a SOMO, an acronym for *singly occupied molecular orbital*. The hydroxyl radical HO• is a good example of this second possibility. Most radicals

are highly unstable and are responsible for many deadly chain reactions leading to explosions.

iii) The HOMO is degenerated meaning that the molecule will exist under several spin states depending on the number of electrons that are left as well as the total number of energy levels that are degenerated. Dioxygen O_2 is a typical example of such a situation, with two spin states: S = 0 (singlet spectroscopic state) and S = 1 (triplet spectroscopic state) linked to a doubly degenerated SOMO. Owing to Hund's rule, the state of the lowest energy is the triplet, noted with the spin multiplicity (2s+1) as a superscript before the formula: $^3O_2$. As dihydrogen H_2 and water H_2O are singlet state molecules, the direct oxidation of hydrogen by oxygen (total spin S = 0 + 1 = 1) is thus spin forbidden (final state: water with spin S = 0) and cannot spontaneously occur.

INTERNAL ENERGY, HEAT AND WORK

It is crucial realizing that there is absolutely no room for such a thing called *heat* at a microscopic level (atoms and molecules). Accordingly, if there are quantum operators for position in space, energy, linear and angular momenta and associated conservation laws arising from Noether's theorem, it is not possible defining quantum operators for heat and time. Consequently, there is no reason for heat to be a conserved entity in full agreement with Count Rumford's cannon boring experiments. Similarly, as there is no quantum operator for time, the origin of time cannot remain undetermined and arbitrary as soon as heat exchanges becomes allowed. Heat and the arrow of time (irreversibility) are thus two deeply entangled notions rendering meaningless the assimilation of heat with a particular form of energy. Heat is in fact an alien concept to energy and as metabolism is a friend concept of heat it logically follows that metabolism and life are alien concepts to energy. Moreover, adding heat and work in order retrieving a conserved total internal energy state function as usually done in expressing the first law of thermodynamics, should as already stressed, be avoided. It follows that adding a label "internal" to the word "energy" means something else that ought to be further clarified and discussed.

A perplexing thing is obviously that the new concept of internal energy shares with mechanical energy the same physical unit (joules J) despite the fact of being of a fundamentally different nature. In fact, the slipping from *mechanical energy* to *internal energy* is the consequence of considering not a single quantum entity, but rather a huge number (typically 10^{24}) of indistinguishable quantum entities. This means switching from the microscopic world of atoms and molecules to the macroscopic world of substances with the imperative need of distinguishing between *microstates* and *macrostates*. Accordingly, for a system made of N particles, a microstate is the enumeration of 6N numbers specifying the spatial positions (x_i, y_i, z_i) and velocities (v_{xi}, v_{yi}, v_{zi}) of each particle (i = 1,…, N) belonging to the considered system. For the same system, a macrostate is an arbitrary set of n control variables such as: temperature, pressure, electrical potential, chemical potentials, electric field, magnetic field, surface tension, altitude, speed of the center of mass, etc. For a pure neutral substance at rest without boundaries and not submitted to gravitational, electric or magnetic fields, a macrostate is defined by only 2 variables: temperature and pressure against 6N for each microstate. Temperature is necessary to know what will be the highest energy level (n_{max}) accessible in the $\{\varepsilon_n\}$ energy spectrum putting a constraint on microstates' velocities (v_{xi}, v_{yi}, v_{zi}), while pressure is necessary to put a constraint on allowed microstates' positions (x_i, y_i, z_i). As each particle of a microstate may be found under different excited states $\{\varepsilon_1, \varepsilon_2, \ldots, \varepsilon_{nmax}\}$, one may define the macroscopic total energy, also called internal energy as:[24]

$$E_{int} = \sum_i n_i \cdot \varepsilon_i \Rightarrow dE_{int} = \sum_i dn_i \cdot \varepsilon_i + \sum_i n_i \cdot d\varepsilon_i$$

A comparison between expressions of E_{molec} and E_{int} is quite instructive and clearly shows the difference between molecular energy, a concept whose value depends only on occupancy numbers (v_n = 0, 1 or 2) and internal energy which is a statistical concept whose value is fixed by populations n_i (i = 0, 1, …, +∞) of each accessible energy levels ε_i.

Now, at the thermodynamic level, it was recognized that if a system is thermally isolated from its surroundings (no exchange of heat, *i.e.* q = 0) and also mechanically isolated (no work is done, *i.e.* w = 0), then the function E_{int} of its thermodynamic state does not change. That is one fundamental property that the mechanical energy E and the internal energy E_{int} have in common. The second is that if the mechanical system is not isolated, its total energy E is not a constant of the motion, but can change, and does so by an amount equal to the work done on the system: ΔE = w. Likewise, in thermodynamics, if a system remains thermally insulated (q = 0), but is mechanically coupled to its environment, which does work w on it, then its internal energy E_{int} changes by an amount equal to that work: ΔE_{int} = w. This coincidence of two such fundamental properties is what led to the hypothesis that the thermodynamic function E_{int} has

something to do with the mechanical energy E, the total of the kinetic and potential energies of the molecules, of a system having huge number of degrees of freedom.

But a critical assumption, thermal insulation, remains for identifying E with E_{int}, as if the system is not isolated, exchanging heat with its surroundings for instance, then the energy E is no more a constant of the motion. It is precisely at this point, that a divorce occurs between thermodynamic energy and mechanical energy, and one should thus refrain from writing E_{int} = q + w, something allowed on the ground that q and w share the same physical unit (Joules), but that is nevertheless forbidden on the ground that mechanical energy (work) has an associated quantum operator, whereas it exists no quantum operator associated to heat. Deeply linked with this divorce is the distinction between reversible and irreversible phenomena. This divorce is also the reason why Max Planck about a hundred years ago was complaining against an error "impossible to eradicate" concerning the confusion made by scientists between mechanical, thermodynamic and Carnot reversibility.[25] These three kinds of reversibility may be clarified by considering a system A evolving into another B. At the level of microstates, reversibility means the reversal of all constituent parts velocities, to carry back the system to state A along its previous followed path. But, to restore the original state A, a second reversal of all velocities is necessary when each individual part has recovered its initial position. This is the so-called mechanical reversibility. But, one may also envision running the system is the opposite direction B → A, restoring only the original *macrostate* in terms of temperature and pressure for instance (Carnot's reversibility) and not the original *microstate* (mechanical reversibility). However, it may happen that the reverse B → A process at a macrostate level may not be feasible owing to supercooling at a phase transition for instance. Nevertheless, if the original macrostate could be recovered by a succession of states B → C → D → A, without any external changes, then we are facing thermodynamic reversibility.

But nowadays, who cares about all these fundamental distinctions? Confusion between mechanical and thermodynamic reversibility leads immediately to the apparent impossibility of reconciling the second law, claiming the existence in nature of irreversible processes, with the full reversibility of the equations of motion. But if one makes the distinction between a mathematical fact (mechanical reversibility impossible to realize on a huge amount of constituent parts) and what can be really done in a laboratory (thermodynamic reversibility), the apparent paradox disappears.

ENTROPY AND IRREVERSIBILITY

After this digression into quantum physics, showing that heat cannot be a form of energy but something else, we may go back to Kelvin's expression of Carnot's principle. The key point is that this principle is formulated through an inequality, the equality holding only for a reversible transformation. Kelvin could not go one step further by introducing a new state function S such that for a sum of infinitesimal heat increments dQ along a cycle where the end state coincide with the initial state:

$$\oint \frac{dq}{T} \leq 0 \Rightarrow \int_A^B \frac{dq}{T} \leq S_B - S_A$$

Again, the equal sign applies if and only if the process A → B is reversible. Here, T denotes the temperature of a heat bath with which the system is momentarily in contact to exchange heat, which is not necessarily the temperature of the system. It was the German physicist Rudolf Clausius that was responsible for this crucial step having coined the name "entropy" for this quantity (meaning "in evolution" through heat), by analogy with the word "energy" (meaning in action through work)[26]. One may notice that in such a relationship, the negative of the left-hand side may be interpreted as the entropy gained by the heat reservoirs that constitute, for the system, the "rest of the universe". So for two processes that begins and ends in thermal equilibrium, a golden rule for evolution with heat involvement should be:

$$S(\text{final}) \geq S(\text{initial}) \Leftrightarrow \Delta S_{univ} = S(\text{final}) - S(\text{initial}) \geq 0$$

Such an inequality means that only three kinds of processes have to be considered in nature[27]:
i) Natural or irreversible process: $\Delta S_{univ} > 0$.
ii) Idealized or reversible process: $\Delta S_{univ} = 0$.
iii) Unnatural or non-spontaneous process: $\Delta S_{univ} < 0$.

It is worth noting that such a formulation involving the universe, a spherical entity having a diameter of about 880 Ym, is mandatory as it is the only really closed system unable to exchange matter, heat or radiation with its surroundings. Consequently, an implicit mandatory act is to split the universe total entropy change ΔS_{univ} into a first term ΔS_{syst} summing all changes occurring in one part of the universe of particular interest called the "system", and another sum of all entropy change ΔS_{surr} occurring in the remaining part, called the "surroundings". It is worth noting that such a partition is totally arbitrary, as it exists nothing in physics that would allow declaring that such one given partition is better than another partition.

But, having to deal with the whole universe whose diameter is 880 Ym may be a really shocking situation for a meter-sized scientists and worst for a micrometer-sized bacteria. The only scientist that would have not been shocked would probably be the German physicist Ernst Mach who was convinced that local physical laws are determined by the large-scale structure of the universe. Thus speaking of the law of inertia, Mach's own words were:

> *When, accordingly, we say that a body preserves unchanged its direction and velocity in space, our assertion is nothing more or less than an* ***abbreviated reference to the entire universe****... In point of fact, it was precisely by the consideration of the fixed stars and the rotation of the earth that we arrived at knowledge of the law of inertia as it at present stands, and without these foundations we should never have thought of the explanations here discussed. The consideration of a few isolated points,* ***excluding the rest of the world, is in my judgment inadmissible.***[28]

It is worth recalling that Mach's book was highly influential in orienting Albert Einstein thoughts towards formulation of its theory of general relativity that requires an ether connecting every mass:

> *Recapitulating, we may say that according to the general theory of relativity space is endowed with physical qualities; in this sense, therefore,* ***there exists an ether****. According to the general theory of relativity* ***space without ether is unthinkable****; for in such space there not only would be no propagation of light, but also no possibility of existence for standards of space and time (measuring-rods and clocks), nor therefore any space-time intervals in the physical sense.*[29]

We have put in bold character some crucial words such as inadmissible or unthinkable in the mouth these two top scientists that both suggest that there is great danger in believing that isolated masses may exist. For Mach, the mere fact that two masses mutually interact is the consequence of the existence of the whole universe. Similarly, for Einstein, the same two masses can never be disconnected from the unique ether filling the whole universe.

Such considerations are crucial for biology in realizing that it is meaningless of speaking of a living cell without speaking of what surrounds this living cell. Similarly, in chemistry, it is the existence of a container that allows speaking of a chemical bond between atoms. Atoms and molecules exist only because they are confined in a small part of the whole universe. A proof that chemical bonds have no existence by themselves is clearly evident by letting a molecule diffuse into the intergalactic space. Here the volume is so huge that the molecule will spontaneously dissociate into atoms and that atoms will also separate into protons, neutrons and electrons, whatever the considerable "attractive forces" holding these particles together on earth. Nuclei, atoms and molecules can manifest themselves only after confinement into a small volume (nucleus for nucleons and atoms or molecules for electrons). This is precisely why the unique state of matter in the universe is the plasma state and why any atmosphere around a planet becomes an ionosphere at its interface with intergalactic space. In other words, what we see at a local scale cannot be disconnected from configurations of matter at much larger scale. Such a fundamental fact of nature is evident not only in classical mechanics (law of inertia), general relativity (existence of an ether connecting all masses) but also in quantum physics where it could also be demonstrated that molecular structures have no intrinsic existence[30]. If such implicit subtleties are evident for scientists well acquainted with general relativity or quantum mechanics, they are just ignored by other scientists not trained into these two disciplines, prone to believe that atoms or molecules have an existence independent of their container. Being ignorant that atoms and molecule are just ideas or conceptual schemes that have no independent reality has led to many paradoxes and confusing situations in science. In fact, the only real tangible thing is the universe taken as a whole that constitutes the single and only acceptable reference state for defining fictive entities such as atoms, molecules, cells, planets and galaxies as lucidly perceived by Ernst Mach. Such a view agrees fully with quantum mechanics, as the only way for having null wave functions is to go at the farthest edge of the universe. Obviously, people trained to consider that matter particles are submitted to local forces may be deeply shocked by such an effect of the configuration of the whole universe on tiny little things such as molecules or cells. But, realizing that forces in fact does not exist being just the effect of non-local fields filling the whole universe, the shocking statement becomes a mere platitude, an obvious consequence of modern ideas about space, time and matter.

Forgetting that the only real thing is the whole universe was responsible, in thermodynamics, for the assimilation of heat with energy. By putting focus exclusively on energy that can never change, entropy, the only concept allowing evolution with time, was then assimilated to disorder and chaos. So, one should first realize that heat is not a particular form of energy, but is rather the manifestation of an entropy flow. Another crucial point is that entropy is not a measure of disorder but a

quantity like mass, amount of motion, volume, electrical charge, area, particles that may be exchanged between two systems. So, when system A accepts entropy from system B, temperature T_A increases (heating), volume V_A increases (expansion) and the so-called "bonds" between sub-parts are destroyed increasing the total number of particles N_A (disaggregation, loss of structure, catabolism in biology). Of course sub-system B that have given entropy to A has decreased its temperature T_B (cooling), occupies a smaller volume (contraction) and has created new "bonds" decreasing its total number of particles N_B (aggregation, creation of structure, anabolism in biology). Most importantly, if the entropy exchange is irreversible, this means that *de novo* entropy has also been created whose excess has been released in the universe to which systems A and B belong. At this fundamental level there is not need bothering about energy because the total sum (including the energy stored in the universe) is the same before and after the exchange of entropy (Noether's theorem). So, the real tangible thing allowing perceiving an arrow of time should be entropy. And here, we are not speaking of the entropy content of a sub-system, but of the entropy of the universe, taken as wholeness.

FIRST AND SECOND LAW OF THERMODYNAMICS

However, Clausius's claim for the existence of a thing called entropy has the drawback to put at the root of thermodynamics two very different laws: the first law emphasizing conservation of something identified with energy ("*Die Energie der Welt bleibt constant*") and the second law introducing entropy, associated to heat that is doomed to never decrease ("*Die Entropie der Welt strebt einem Maximum zu*"). Moreover, enunciating the first law as equivalence between work (a conserved entity) and heat (something that could be created) has the consequence of rendering completely obscure the meaning of entropy, by assigning its attributes to energy, a conserved quantity. As a result entropy is reduced to a lifeless empty shell with obscure physical meaning while heat assumes a schizophrenic double role that is to say a strange mixture of energy and entropy, instead of being clearly considered as caused by an entropy flow.[19]

If one insists on speaking of energy and introduce correctly the first law, the only correct way is to follow the mathematician Constantin Caratheodory that distinguishes between adiabatic processes (no heat exchanged) and non-adiabatic processes (heat exchange are allowed)[31]. Next, experiments demonstrate that adiabatic work of a given quantity produces the same change in temperature no matter how the work is produced, whether by friction, by turbulent motion, by compression of gas, or electrically. Then, because the adiabatic work is independent of the kind of work that is done, it should be equal to the difference between two values of a state function $U = E_{int}$, the *internal energy*, so that the energy change is defined in differential form as $dU = \delta w(\text{adiabatic})$, where δ is used for work because it is a state function only for adiabatic changes and not for any kind of change as U. Consequently, if a change of state is not carried out adiabatically, the work δw is no longer equal to dU and the *numerical* difference between dU and δw is attributed to the transfer of a certain amount of heat $\delta q = T \cdot dS$ (*i.e.* transfer of entropy) to or from the surroundings as a result of a difference of temperature across a thermally conductive boundary. As heat is not an exchange of energy, but an exchange of entropy, one should refrain to write that $\delta q = dU - \delta w$ as usually done, but rather that $dU(\text{non-adiabatic}) \neq \delta w(\text{adiabatic})$.

The identification $dU = \delta w(\text{adiabatic})$ applies in fact only for systems having a constant volume ($dV = 0$). For systems evolving at constant pressure ($dP = 0$), the effective work available under adiabatic conditions is reduced by a quantity $-P \cdot dV$ that corresponds to the work done by the system against the applied pressure when the total volume changes by an infinitesimal quantity dV, leading to $dU = \delta w(\text{adiabatic}) - P \cdot dV = \delta w(\text{adiabatic}) - d(PV)$. The second expression stems from the fact that $dP = 0$, allowing introducing a new state function $H = U + P \cdot V$, named *enthalpy*, and such that $dH = \delta w(\text{adiabatic})$.

Concerning the second law, existence of entropy means that a natural representation of internal energy is to consider this entity as a function of three extensive variables: entropy S, volume V and number of particles N:

$$dU(S,V,N) = \left(\frac{\partial U}{\partial S}\right)_{V,N} dS + \left(\frac{\partial U}{\partial V}\right)_{S,N} dV +$$

$$\left(\frac{\partial U}{\partial N}\right)_{S,V} dN = T \cdot dS - P \cdot dV + \mu \cdot dN$$

These makes appear, temperature T, pressure P and chemical potential μ as intensive conjugated variables to entropy, volume and number of particles. Now, let's suppose that X is a conserved quantity for a system divided into sub-systems A and B. As $X_A + X_B = X_{tot}$ is fixed, we should have for any transfer of X between A and B: $dX_{tot} = 0$, i.e. $dX_A = -dX_B$. But we know from Clausius' second law that at equilibrium the total entropy $S_{univ} = S_A + S_B$ tends to be maximized, meaning that:

$$\frac{\partial S_{univ}}{\partial X_A}=\frac{\partial S_A}{\partial X_A}+\frac{\partial S_B}{\partial U_A}=\frac{\partial S_A}{\partial X_A}-\frac{\partial S_B}{\partial X_B}=0 \Rightarrow \frac{\partial S_A}{\partial X_A}=\frac{\partial S_B}{\partial X_B}$$

$$dS_{univ}=\frac{\partial S_A}{\partial X_A}\cdot dX_A+\frac{\partial S_B}{\partial X_B}\cdot \delta X_B=\left(\frac{\partial S_A}{\partial X_A}-\frac{\partial S_B}{\partial X_B}\right)\cdot dX_A \geq 0$$

But X could well be the total energy U(S,V,N) meaning that:

$$\left(\frac{\partial S}{\partial U}\right)_{N,V}=\frac{1}{T}\Rightarrow dS_{univ}=\left(\frac{1}{T_A}-\frac{1}{T_B}\right)\cdot dU_A \geq 0$$

Consequently, for $T_A < T_B$, one should have $dU_A > 0$, stating that heat must flow from the high temperature sub-system towards the low-temperature one (thermal transfer). But if X stands for the total volume V(U,S,N), we have by the same reasoning:

$$dV(S,U,N)=\left(\frac{\partial V}{\partial S}\right)_{U,N}dS+\left(\frac{\partial V}{\partial U}\right)_{S,N}dU+$$

$$\left(\frac{\partial V}{\partial N}\right)_{S,U}dN=\frac{T}{P}\cdot dS-\frac{dU}{P}+\frac{\mu}{P}\cdot dN$$

$$\left(\frac{\partial S}{\partial V}\right)_{N,U}=\frac{P}{T}\Rightarrow dS_{univ}=\left(\frac{P_A}{T_A}-\frac{P_B}{T_B}\right)\cdot dV_A \geq 0$$

Then, at constant temperature ($T_A = T_B$) and $P_A > P_B$, one should have $dV_A > 0$, stating that volume should flow from the low-pressure sub-system towards the high-pressure one. A last possibility could be that X is the total number of particles N(U,S,V):

$$dN(S,U,V)=\left(\frac{\partial N}{\partial S}\right)_{U,V}dS+\left(\frac{\partial N}{\partial U}\right)_{S,V}dU+$$

$$\left(\frac{\partial N}{\partial V}\right)_{S,U}dV=-\frac{T}{\mu}\cdot dS+\frac{dU}{\mu}+\frac{P}{\mu}\cdot dV$$

$$\left(\frac{\partial S}{\partial N}\right)_{U,V}=-\frac{\mu}{T}\Rightarrow dS_{univ}=\left(\frac{\mu_B}{T_B}-\frac{\mu_A}{T_A}\right)\cdot dN_A \geq 0$$

Thus, at constant temperature ($T_A = T_B$,) and $\mu_A < \mu_B$, one should have $dN_A > 0$, stating that transport of particles is required from the high chemical potential sub-system towards the low chemical potential one (diffusion). It also follows from the above reasoning that if two systems are in thermal, mechanical as well as diffusive equilibrium, temperatures, pressures as well as chemical potentials of both systems must be the same everywhere in both systems. So, we see that through the idea of maximizing entropy, it has been possible to give a precise definition of the so-called intensive variables T, P, μ as conjugate variables of the three extensive variables of a state function U(S, V, N). It is worth noticing that no special meaning has been here given to the fact that according to the first law U should be a conserved quantity because if one has U(S, V, N) it also logically follows that one also has S(U, V, N) or V(S, U, N) as well as N(S, U, V). In other words, internal energy U, entropy S, volume V or total number of particles N, are all good state variables of any system. This means that staying at a macrostate level, there is no clear reason to favor energy over entropy, volume or number of particles. Accordingly, under extrapolation at the scale of the universe, saying that energy should always be conserved is fully equivalent to the statement that the total volume of the universe should remain the same or to the statement that it is not allowed to create or destroy particles. Putting emphasis on energy and not on entropy, volume or number of particles is at this level just not admissible.

There is also a concern by writing the first law as dU(S,V,N) = T·dS – P·dV + μ·dN because such an expression cannot tell us what will happen if our system bears a total electric charge Q, another extensive variable not appearing in the definition of U. Accordingly, it will be totally ridiculous to speak of a living cell as U(S,V,N) system because without electrical potentials ψ created by ions there would be no life. Fortunately, in our formulation of what is internal energy we have complete freedom for defining what is variable X. Let's for instance assume that X is electrical charge Q, then all we have to do is to add a new electrical term for defining the internal energy variation: dU(S, V, N, Q) = T·dS - P·dV + μ·dN + ψ·dQ and it immediately follows that:

$$dQ(S,\ldots)=\left(\frac{\partial Q}{\partial S}\right)_{\ldots}dS+\cdots=-\frac{T}{\psi}\cdot dS+\cdots\Rightarrow\left(\frac{\partial S}{\partial Q}\right)_{\ldots}=-\frac{\psi}{T}\Rightarrow dS_{univ}=\left(\frac{\psi_B}{T_B}-\frac{\psi_A}{T_A}\right)\cdot dQ_A$$

Then, at constant temperature ($T_A = T_B$,) and $\psi_A < \psi_B$, one should have $dS_{univ} \geq 0$ or $dQ_A > 0$. This means that positive electrical charge has to flow from the high electric potential sub-system towards the low electrical potential one with, at equilibrium, the same electrical potential everywhere in the system. Alternatively, one may also say that negative electrical charge has to flow from the low electrical potential sub-system towards the high electrical potential one. But these considerations apply only to a cell with static free electrical charges. What about the displacement of bound charges after application of an electric field E? To take into consideration possible changes in the total dipolar moment D

(C·m), we may write dU(S, V, N, Q, D) = T·dS - P·dV + μ·dN + ψ·dQ + E·dD, meaning that:

$$dD(S,\dots)=\left(\frac{\partial D}{\partial S}\right)_{\dots} dS+\dots=-\frac{T}{\mathrm{E}}\cdot dS+\dots\Rightarrow\left(\frac{\partial S}{\partial D}\right)_{\dots}=$$

$$-\frac{\mathrm{E}}{T}\Rightarrow dS_{univ}=\left(\frac{\mathrm{E}_B}{T_B}-\frac{\mathrm{E}_A}{T_A}\right)\cdot dP_A$$

Then, at constant temperature ($T_A = T_B$,) and $E_A < E_B$, one should have $dS_{univ} \geq 0$ or $dP_A > 0$. This means that some dipolar moment should flow from the high electric field sub-system towards the low electric field one with, at equilibrium, the same electric field everywhere in the system. But we are still not considering a real living cell because free charges may also move generating magnetic fields B. We are thus also led to consider possible changes in a the total magnetic moment M ($A{\cdot}m^2$), by adding a new variable to the first law dU(S, V, N, Q, D, M) = T·dS - P·dV + μ·dN + ψ·dQ + E·dD + B·dM, meaning that:

$$dM(S,\dots)=\left(\frac{\partial M}{\partial S}\right)_{\dots} dS+\dots=-\frac{T}{\mathrm{B}}\cdot dS+\dots\Rightarrow\left(\frac{\partial S}{\partial M}\right)_{\dots}=$$
$$-\frac{\mathrm{B}}{T}\Rightarrow dS_{univ}=\left(\frac{\mathrm{B}_B}{T_B}-\frac{\mathrm{B}_A}{T_A}\right)\cdot dM_A$$

Again, at constant temperature ($T_A = T_B$,) and $B_A < B_B$, one should have $dS_{univ} \geq 0$ or $dM_A > 0$. This means that magnetic moment is expected to flow from the high magnetic field sub-system towards the low magnetic field one with, at equilibrium, the same magnetic field everywhere.

One may thus begin understanding that the first law of thermodynamics is not really a law, but rather a mere kitchen recipe for dealing with many kinds of perturbations. Suppose for instance that we apply a perturbation that is not thermal, mechanical, chemical, electrical nor magnetic. Then the first "law" stating the conservation of the function U(S, V, N, Q, D, M) will of course be violated because energy could now flow in a reservoir not explicitly considered in the total internal energy. In other words, the first "law" will have to lose its status of being a fundamental law of nature. In fact, this will never happen because the first "law" is a clever recipe allowing dealing with anything you want to deal with. Accordingly, for a living cell it should be obvious that at least one variable is still missing in the U(S, V, N, Q, D, M) state function. Until now, we have not given a single clue about how distinguishing between sub-systems A and B. This is because we are just playing a purely mathematical game with a recipe U(S,…) associated to the maximization of the S parameter. If we want to consider a real system such as a living cell, one have to say something about the area A of the physical interface separating the cell from its surroundings by writing: dU(S, V, N, Q, D, M, A) = T·dS - P·dV + μ·dN + ψ·dQ + E·dD + B·dM + σ·dA, where σ is the interfacial tension responsible for changes in area:

$$dA(S,\dots)=\left(\frac{\partial A}{\partial S}\right)_{\dots} dS+\dots=-\frac{T}{\sigma}\cdot dS+\dots\Rightarrow\left(\frac{\partial S}{\partial A}\right)_{\dots}=$$

$$-\frac{\sigma}{T}\Rightarrow dS_{univ}=\left(\frac{\sigma_B}{T_B}-\frac{\sigma_A}{T_A}\right)\cdot dM_A$$

It may then be anticipated that at constant temperature ($T_A = T_B$,) and $\sigma_A < \sigma_B$, one should have $dS_{univ} \geq 0$ or $dA_A > 0$. This means that area should flow from the high interfacial tension sub-system towards the low interfacial sub-system with, at equilibrium, the same interfacial tension everywhere.

For a real living cell, one may also notice that life has appeared on Earth and that this planet through its total mass M and radius R creates a gravitational field g = G·M/R, where G is Newton's universal gravitational constant. As a real living cell is composed of N particles having masses, the total weight W = m·g, should be an additional extensive variable for the internal energy associated to altitude h a conjugate intensive one: dU(S, V, N, Q, D, M, A, W) = T·dS - P·dV + μ·dN + ψ·dQ + E·dD + B·dM + σ·dA + h·dW, leading to a new equilibrium condition in presence of gravity:

$$dW(S,\dots)=\left(\frac{\partial W}{\partial S}\right)_{\dots} dS+\dots=-\frac{T}{\mathrm{h}}\cdot dS+\dots\Rightarrow$$
$$\left(\frac{\partial S}{\partial W}\right)_{\dots}=-\frac{\mathrm{h}}{T}\Rightarrow dS_{univ}=\left(\frac{\mathrm{h}_B}{T_B}-\frac{\mathrm{h}_A}{T_A}\right)\cdot dW_A$$

With the law $dS_{tot} \geq 0$ it may be anticipated that at constant temperature ($T_A = T_B$,) and $h_A < h_B$, one should have $dW_A > 0$. This means that masses should flow from the high altitude sub-system towards the low altitude one with, at equilibrium, the same altitude for all weights.

The advantage of such a formulation of thermodynamics is that whatever your definition of what is a macrostate the "conserved" internal energy U in terms of variables (S, V, N, Q, D, M, A, W,…), evolution is always ruled by a single fundamental law: $dS_{univ} \geq 0$ with transfer of entropy, volume, particles, electrical charge, dipolar moment, magnetic moment, area or masses ruled by an intensive parameter measuring a kind of "energy concentration" (temperature, pressure, chemical or electri-

cal potential, electric or magnetic field, surface tension, altitude, etc…). The three dots in the above formulations means "*any quantity that doubles when the amount of a given stuff is doubled*" for extensive variables and "*corresponding energy concentration associated to a given stuff*" for intensive variables. And of course, it exists an infinite number of stuffs with an infinite number of ways of measuring energy concentration relative to a given stuff. For instance, if you consider that the center of mass of a living cell has a speed v_{CM} (intensive energy concentration) the associated extensive stuff will be the amount of motion of this center of mass p_{CM} of the cell with $dU = \ldots + v_{CM} \cdot dp_{CM}$.

The quite fuzzy mongrel aspect of energy was indeed well perceived by the French mathematician Henri Poincaré:

> *In every particular case we clearly see what energy is, and we can give it at least a temporary definition; but it is impossible to find a general definition of it. If we wish to enunciate the principle in all its generality and apply it to the universe, we see it vanish, so to speak, and nothing is left but this — there is something which remains constant.*[32]

This is why, as far as life phenomenon is concerned, one should not rely on energy and the first law, but only on the second law stating that for any kind of evolution a single non-ambiguous and universal criterion should be used: $dS_{univ} \geq 0$. In fact, it should be easy to realize that as evolution means that it exists a stuff called "time" that is always flowing from past to future, time and the second law are in fact two different ways of speaking of the same basic stuff of our universe.

ENTROPY AND MACROSTATE MULTIPLICITY

So, among all the possible extensive variables that could be associated to a macrostate, entropy and not energy should be the privileged one because it is the only variation that is allowed to change in a unique direction defining unambiguously a biological time for any living species. Unfortunately, this logical choice has not been retained by biology that focuses exclusively on the extensive fuzzy variable: energy. Such a wrong choice is beyond any doubts linked to the fact that modern science is born after identification of the force concept during the eighteenth century through the birth of Newtonian's mechanics. The next step logical step was to move during the nineteenth century from forces ($M \cdot L \cdot T^{-2}$) that may appear or disappear to something that could never be created nor destroyed (first law), *i.e.* energy ($M \cdot L^2 \cdot T^{-2}$). If this was a quite interesting move for understanding the behavior of inert matter, it was a complete sterile move for a good comprehension of living systems that are doomed to be born, to perpetuate (life) and finally to die. Even if energy and entropy were born the same year (1854) from the study of heat engines, entropy has been perceived from the very beginning as a negative "bad" thing, *i.e.* a degraded form of energy that is inexorably dispersed through the whole universe and that could never be recovered for performing useful work.

Fortunately, through the advances made in kinetic gases theory, it was realized that temperature, the conjugate intensive parameter of entropy could be associated to the average kinetic energy of a large assembly of tiny particles that could not be cut into smaller pieces through chemical means (atoms). Similarly, pressure that is the conjugate intensive parameter of volume could be associated to the average force per unit area exerted by atoms hitting the walls of a container. This was the birth of statistical physics that soon leads Ludwig Boltzmann to give a microscopic interpretation of the "bad guy" preventing heat engines to work with 100% efficiency: $S = k_B \times \ln \Omega$. It is worth noting that k_B, the so-called "Boltzmann's constant" was not introduced by Boltzmann itself, but by Max Karl Ernst Ludwig Planck that was deeply interested in – even obsessed with – the second law of thermodynamics. The constant was introduced with another fundamental constant, the quantum of action h (also named Planck's constant) for explaining the mathematical form of the black body radiation spectrum[33]. In this relationship Ω is called the macrostate's multiplicity, that is to say the total number of microstates (positions and velocities of all particles constituting the system) compatible with a given macrostate. Since the logarithm is a monotonic function, the tendency of multiplicity Ω to increase is the same thing as saying that entropy tends to increase: $\Delta S_{univ} \geq 0$. Another advantage of such a formulation is that considering our two sub-systems A and B, one has $\Omega_{tot} = \Omega_A \times \Omega_B$ and thus $S_{tot} = S_A + S_B$, the familiar extensive property of entropy.

The power of this new formulation of entropy may be easily demonstrated by considering a system of N distinguishable particles placed in a volume V at temperature T. From quantum physics, we know that it is possible to associate to each particle of mass m, a DeBroglie's thermal wavelength:

$$\Lambda = \frac{h}{\sqrt{2\pi m k_B T}}$$

Consequently, at this temperature each particle occupies a quantum volume $v = \Lambda^3$, cutting the total vol-

ume into $Z = V/\Lambda^3$ elementary cells. Therefore, there are $\Omega = Z^N$ equivalent ways to spread the N distinguishable particles over Z elementary cells, leading to an entropy:

$$S = k_B \times \ln \Omega = Nk_B \times \ln\left(\frac{V}{\Lambda^3}\right) \propto \mathrm{N}k_B \times \ln\left(V \cdot T^{3/2}\right)$$

We thus learn from Boltzmann's equation that entropy increases for any increase inthe total number of particles N, of the available volume V and of the temperature T. In fact, the above relationship is not quite correct because quantum physics imposes that atoms and molecules are indistinguishable particles. The computation of the multiplicity Ω in such a case is trickier and the correct result is:[34]

$$S = Nk_B \times \ln\left(\frac{V}{N\Lambda^3}\right) \propto \mathrm{N}k_B \times \ln\left(\frac{V \cdot T^{3/2}}{N}\right)$$

Now, for an isochoric process in a closed system characterized by $\Delta N = \Delta V = 0$, it comes that $\Delta S = Nk_B \cdot \ln(T_f/T_i)^{3/2}$, while for an isothermal process ($\Delta N = \Delta T = 0$) we have $\Delta S = Nk_B \cdot \ln(V_f/V_i)$. This demonstrates, without any reference to the first law, that the sole knowledge of entropy is sufficient to understand the basic behavior of a system of N particles enclosed in a volume V at temperature T. We may also predict that for an isentropic process ($\Delta S = \Delta N = 0$), any expansion ($\Delta V > 0$) should be associated to a decrease in temperature. Introducing now the first law stating that for a mono atomic ideal gas, $U = (3/2)Nk_B \cdot T$, the derivation of the ideal gas law is straightforward:

$$S \propto \mathrm{N}k_B \times \ln(V) \Rightarrow \left(\frac{\partial S}{\partial V}\right)_{N,U} = \frac{P}{T} \Rightarrow \frac{P}{T} = \frac{Nk_B}{V} \Leftrightarrow$$

$$P \cdot V = Nk_B \cdot T$$

It then follows that for an isobaric process in a closed system ($\Delta P = \Delta N = 0$), we should have $P/Nk_B = T/V = cste$, meaning that $\Delta S = Nk_B \cdot \ln (V_f/V_i)^{5/2}$. Considering again an isochoric process, we have $V = Nk_BT/P = cste$, meaning that $\Delta S = Nk_B \cdot [\ln (T_f/T_i)^{5/2} - \ln (P_f/P_i)]$, while for an isothermal one $T = P \cdot V/Nk_B = cste$, leading to $\Delta S = Nk_B \cdot [\ln (V_f/V_i)^{5/2} - \ln (P_f/P_i)^{3/2}]$.

So, through the simple equation $S = k_B \cdot \ln \Omega$, many predictions could be made that could all be confirmed by making experiments with gases. Even the second law $dS_{univ} \geq 0$ could be anticipated by considering that if Ω_A is the multiplicity of a macrostate A and Ω_B is the multiplicity of another macrostate B of the same system, the most probable macrostate should be the one displaying the largest multiplicity, *i.e.* the largest entropy. A microstate might be inaccessible because it has the wrong energy. So, from a statistical viewpoint, the second law means that states always evolve from configurations of low probability (small multiplicity) towards configurations of maximum probability (the highest possible multiplicity compatible with the imposed constraints). Again, it is worth noting that concepts such as energy, heat or work introduced for dealing with heat engines are completely absent from this formulation. Moreover, associating energy with Hamiltonian or Lagragian operators or functions is surely quite interesting but totally useless as far as thermodynamics is concerned.

To reconcile both approaches, one should use a thermostat that fixes the temperature T and thus puts a constraint on the average quadratic speeds of the constituent parts. This allows mechanical energy to fluctuate at a microstate level with no important consequences for the macrostate level. This stems from the fact that fluctuations in the energy are minute compared with the total energy of the thermostat. In such a case, the internal energy U of a system of fixed temperature T may be identified to the average single particle mechanical energy about which the system's mechanical energy fluctuates:

$$\beta = \frac{1}{k_B T} \Rightarrow Z(\beta) = \sum_i \exp\left(-\frac{\varepsilon_i}{k_B T}\right)$$

$$p_i = \frac{1}{Z} \cdot \exp(-\beta \times \varepsilon_i) \Rightarrow S = -k_B \times \langle \ln p_i \rangle = -k_B \sum_i p_i \times \ln p_i$$

$$\langle E \rangle = \sum_i p_i \times \varepsilon_i = \frac{1}{Z} \sum_i \varepsilon_i \cdot \exp\left(-\frac{\varepsilon_i}{k_B T}\right) = -\left[\frac{\partial \ln Z(\beta)}{\partial \beta}\right]_{V,N}$$

To know the system's energy levels ε_i we must know its volume V for constraining the spatial positions and also the total number of molecules N present in the system, for only then is the mechanical system fully defined. The function $Z(\beta)$ is called the *partition function* and is a very useful entity allowing linking accessible energy levels of a system to a macroscopic property, its internal energy $U = N \times \langle E \rangle$.

FREE ENERGIES

It also follows from the definition of the partition function that entropy may also be written:

$$\ln p_i = -\beta \times \varepsilon_i - \ln Z \Rightarrow S = -k_B \sum_i p_i \times \ln p_i = \frac{\langle E \rangle}{T} +$$

$$k_B \times \ln Z$$

$$F(T,V,N) = -N \times k_B T \times \ln Z = U(S,V,N) - T \cdot S$$

This new kind of "energy" corresponds to *Helmholtz's free energy* that is defined in macroscopic thermodynamics, as the Legendre's transform of internal energy U. From F(T,V,N), another Legendre's transform leads to *Gibbs' free energy*:

$$G(T,P,N) = F(S,V,N) + P \cdot V = H(S,P,N) - T \cdot S$$

In fact, it is possible to derive a more intuitive understanding of what are free energies[35]. Let's consider a set of N molecules able to occupy just two energy levels separated by an energy gap ΔU. To have an equilibrium situation, the number of molecules going from the lower level to the upper level should be at any time equal to the number of molecules going from the upper level to the lower level. According to Boltzmann's law the fraction f of molecules that can be excited to the upper level owing to a thermal fluctuation at constant volume is $f = \exp(-\Delta U/k_B T)$. Now, from the statistical definition of entropy, $S = k_B \cdot \ln \Omega$, where Ω is the multiplicity of a macroscopic state, equilibrium is expected when:

$$\frac{N(up)}{\Omega(up)} = f \times \frac{N(low)}{\Omega(low)} \Rightarrow K_{eq} = \frac{N(up)}{N(low)} = f \times \frac{\Omega(up)}{\Omega(low)} =$$

$$\exp\left[-\frac{\Delta U}{k_B T} + \Delta S\right]$$

Here ΔS is the entropy difference between the two states, ΔS = S(up) – S(low), and K_{eq}, the so-called "equilibrium constant" such that $\Delta F = \Delta U - T \cdot \Delta S = -k_B T \cdot \ln K_{eq}$. Similarly, the fraction f of molecules that can be excited to the upper level owing to a thermal fluctuation at constant pressure would be $f = \exp(-\Delta H/k_B T)$, leading following the same reasoning to the second kind of free energy ΔG = ΔH – T·ΔS. Consequently, if one is interested in populations, the pertinent functions for isothermal transformations are not internal energy U or enthalpy H, but rather the associated free energies F or G depending on the second constrained parameter: volume for F or pressure for P. But what's about considering the case of non-isothermal transformations? It is easy to see by the above reasoning that the pertinent functions for following populations should be $S - \Delta U/k_B T$ at constant volume and $S - \Delta H/k_B T$ and no more ΔF or ΔG that are clearly defined only at constant temperature.

In fact, the same conclusion could be reached by ignoring microstates and considering splitting of the whole universe into system and surroundings separated by an interface that may allow or not entropy exchanges:[36]

$$dS_{univ} = dS_{syst} + dS_{surr} \geq 0$$

As explained above, for micrometer-sized bacteria, universe and surroundings (anything that are not inside the lipid double layer) is really colossal (hundreds of yotta-meters in size) and such a global formulation is not at all adapted to the scale of a cell or of a multicellular organism. But, relying on the fact that energy is a form of adiabatic work δW(adiabatic), *i.e.* a work done with no heat exchange, and that energy cannot be created or destroyed, it is possible to masquerade entropy exchanges with the surroundings as adiabatic work done at a given temperature T:

$$dS_{surr} = \delta W_{surr}(\text{adiabatic})/T = -\delta W_{syst}(\text{adiabatic})/T$$

Moreover, biological transformations usually occur under a constant pressure provided by earth's atmosphere and not with constant volume as living cells may swell or shrink by absorbing or releasing water. Thus introducing enthalpy as $dH = \delta W_{sys}(\text{adiabatic})$, it follows that for any infinitesimal change:

$$dS_{univ} = dS_{syst} - \frac{dH_{syst}}{T_{syst}} \geq 0$$

It is worth noting that such legitimate transformations have completely eclipsed the original partition between the system and its surroundings with a complete palming of the two huge systems (universe and surroundings). We have thus now two equivalent terms: the one at the left dS_{univ} referring explicitly to the whole universe and showing the reason for the second law (no possible decrease of S_{univ}) and the one at the right making only reference to the small sub-system, with a tacit assumption that variations of entropy and enthalpy observed on the system alone are in fact exactly related to entropy variations of the whole universe. In fact such an assumption are usually simply ignored by most scientists not well acquainted with thermodynamic subtleties, giving the false impression that the entropy of the small sub-system has to increase *independently* of the entropy of the whole universe, a major pitfall to be avoided. This was, of course, Schrödinger's first fatal error upon writing his little book about what is life. But the error in forgetting that thermodynamics is the science of the whole universe has still more perverse consequences. Accord-

ingly, if the temperature remains constant during the infinitesimal transformation, then $dT_{syst} = 0$, allowing writing:

$$dS_{univ} = dS_{sys} - \frac{dH_{sys}}{T_{sys}} + H_{sys}\frac{dT_{sys}}{T_{sys}^2} = d\left(S_{sys} - \frac{H_{sys}}{T_{sys}}\right)$$

$$= d\psi_{sys} \geq 0$$

This basically means that at constant pressure and temperature the right criterion of spontaneous evolution is not $dG = d(H - T{\cdot}S) \leq 0$ as usually stated in most textbooks, but rather an increase in the so-called Planck's function $d\psi = d(S - H/T) \geq 0$.[36] One may of course argue that if temperature is constant, $d\psi = -d(G/T) = -dG/T \geq 0$, meaning that as temperature is a positive quantity that $dG = -T{\cdot}d\psi \leq 0$. There is also a deep subtlety here linked to the fact that by writing $dG \leq 0$, one tacitly assume that the system evolves at constant pressure in contact with a thermostat, whereas writing $d\psi \geq 0$ only assume constant temperature whether the system is in contact with a thermostat or not. So, if the criterion $d\psi \geq 0$ is a special case ($dT = 0$) of a most general criterion $dS_{univ} \geq 0$, it also appears the criterion $dG \leq 0$ is a special case of $d\psi \geq 0$ ($dT = 0$ fixed by a thermostat to ensure that both initial and final states are at thermal equilibrium).

The importance of considering $d\psi \geq 0$ and not $dG \leq 0$ as a criterion for spontaneous evolution at constant temperature and pressure is well illustrated by the temperature dependence of the ionization constant of acetic acid[36]. Measurements show that as the temperature is increased from 0 °C, the degree of ionization first increase reaching a maximum just below 25 °C, and then decrease with increasing temperature. But considering the temperature dependence of $\Delta G°$ for this ionization shows a monotonical increase with no maximum in the experimental range of temperatures studied. On the other hand, considering the same temperature dependence of Planck's function $\Delta\psi°$ leads to a dome-shaped curve with a maximum around 25 °C. This demonstrates the clear superiority of Planck's function for comparisons of the degree of spontaneity of a given transformation at different temperatures.[36] Consequently, one should really avoid the common error of thinking that by adding the word "free" before the word "energy", one still refers to energy changes. It should rather be realized that "free energies" are in fact entropies, an obvious statement when looking at Planck's function ψ rather than Gibbs' G. In fact, the error of assimilating Gibbs' free energy to energy may be traced back to 1923 in a very popular thermodynamic treatise.[37] Besides forgetting that thermodynamics is a science of the whole universe, there is also the fact that entropy changes ΔS_{syst} are masqueraded in Gibbs' formulation as energy changes after multiplication by the temperature of the thermostat. Such a manipulation, pushes to the belief for unaware people that a thermodynamic system tries, upon spontaneous evolution, to minimize its energy, as in reality he tries to maximize the entropy of the universe! From this fundamental error follows the wrong idea that changes always proceed from configurations of high energy to that of low energy. In fact, this just cannot be owing to the fact that energy is always conserved, meaning that any energy decrease somewhere must exactly match energy increase elsewhere.[38]

THE SECOND LAW AND THE UNIVERSE

In line with the fact that energy is a conserved quantity that should never created nor destroyed, it may seem at first sight surprising to see molecules with large negative energies popping from zero. In fact, it happens that the decrease in energy is related to a zero energy state where a distance equal to the diameter of the whole universe separates the nuclei from their electrons. This raises the interesting question of what may be the total energy of the whole universe. A pertinent answer would of course be that to have a reasonable chance meeting, nuclei and electrons should have at least some kinetic energy E_{univ} that is different from zero and whose exact value does not really matter. Accordingly, when these particles come close enough to interact, their average kinetic energy increases by a certain amount $\langle\Delta K\rangle = E_{tot} - \langle K\rangle$ due to the trapping of the electrons in nuclei Coulomb's potential (Heisenberg's uncertainty principle: $\Delta p{\cdot}\Delta x \geq \hbar/2$) associated to a decrease in potential energy $\langle\Delta U\rangle = -2\times\langle K\rangle$ (virial's theorem). As total energy should always be conserved, one should have $\langle\Delta K\rangle + \langle\Delta U\rangle = 0 = E_{univ} - 3\times\langle K\rangle$, *i.e.* $E_{univ} = 3\times\langle K\rangle$. There is thus absolutely no decrease in total energy when electronic shells appear around nuclei and when chemical bonds between atoms are created, but just a different partition between kinetic and potential contributions, relative to an arbitrary absolute energy content of the whole universe.

But, if there is the same total energy content between an assembly of separated nuclei and electrons dispersed in the universe and the same assembly occupying a quite tiny volume, why atoms and molecules should form? As explained above, the answer is simply that entropy is higher after formation of atoms and

molecules than before. At first sight, it could be strange associating an entropy increase to a process leading to a strong decrease in volume. But again, the golden rule is that entropy could be allowed to decrease in one small part of the universe (called atoms and molecules), provided that the other parts of this universe have increased their entropy to more than compensate the necessary decrease. And one must not forget that entropy may be associated to visible matter (atoms, molecules) as well as invisible matter (neutrinos) or non-matter (photons). Everything that could be counted as particles (photons, neutrinos, electrons, nuclei, atoms, molecules, cells, organisms, etc.) carries a part of entropy. The higher is the number of entities, the higher the entropy (see above).

Accordingly, as atoms are created in stars and as stars emits a huge number of invisible neutrinos and photons (with a small number that are "visible") in the intergalactic vacuum, a strong increase in the total entropy of the universe is always associated to the formation of nuclei and atoms. In other words if the universe is full of atoms it should also be full of neutrinos and photons. This could be checked by back of an envelope calculation. Let <M> be the average mass of a star (in grams), N_s the total number of stars in a galaxy and N_g the number of galaxies in the universe. The total number of H-atoms should then be $n_H = N_g \times N_s \times <M> \times N_A$, where N_A is Avogadro's constant. Taking the mass of the sun, $m_0 = 2\times10^{33}$ g, as a reference, the stellar and sub-stellar initial mass function (IMF) displays a power law distribution $f(m) = (m/m_0)^{-\alpha}$, with $\alpha = 0.3$ ($m/m_0 \leq 0.08$), $\alpha = 1.3$ ($0.08 \leq m/m_0 \leq 0.5$) and $\alpha = 2.3$ ($m/m_0 \geq 0.5$)[39]. Integration of such IMF being $F(m) = (1/1-\alpha)\times(m/m_0)^{1-\alpha}$ allows computing and averaged mass ratio:

$$\frac{\langle M\rangle}{m_0} = \left[\frac{(m/m_0)^{0.7}}{0.7}\right]_0^{0.08} - \left[\frac{(m/m_0)^{-0.3}}{0.3}\right]_{0.08}^{0.5} + \left[\frac{(m/m_0)^{1.3}}{1.3}\right]_{0.5}^{+\infty} \approx 5.15$$

Now, for a galaxy such as the Milky Way, the total amount of visible mass is $m/m_0 = 0.42\times10^{12}$,[40] leading to an average number of stars $N_s \approx (0.42/5.15)\times10^{12} \approx 0.8\times10^{11}$. Finally, the current best estimate of the total number of galaxies in the universe is $N_g \approx 2\times10^{12}$[41]. So, the total amount of H-atoms in the universe may be estimated as $n_H \approx 2\times10^{12}\times0.8\times10^{11}\times2\times10^{33}\times6\times10^{23} \approx 2\times10^{80}$. For the total number of photons, we may use the black-body equation with a temperature of the cosmic microwave background T(CMB) = 2.726 K[42], as an estimate of the current density of low-energy photons. Converting Planck's black-body function into the phase space number density of photons gives:

$$n(x,p) = \frac{2}{h^3\left[\exp\left(\frac{pc}{k_BT}\right)-1\right]} \Rightarrow N = 8\pi V\left(\frac{k_BT}{hc}\right)^3$$

$$\int_0^{+\infty}\frac{u^2du}{e^u-1} = 16\pi V\times\left(\frac{k_BT}{hc}\right)^3\times\zeta(3)$$

Here $\zeta(3) = 1.202057$ is Apéry's constant, leading to $N(CMB)/V = 411$ photons·cm^{-3}. The volume of the universe being 3.5×10^{86} cm^3, we get a total of 1.44×10^{89} photons of low energy liberated owing to the assembly of all atoms and molecules (including those produced on earth) in the universe. For neutrinos, we have a ratio He/H = 0.075, heavier elements being relatively rare. Given that Helium has two neutrons, and that creating a neutron also creates a neutrino, we can estimate the total number of neutrinos to be about 3×10^{79}. This shows that if neutrinos participate in the overall entropy budget of the universe, photons give, nevertheless, as expected, an overwhelming contribution.

Oblivion of photons' contribution to the entropy budget of the universe has of course deep consequences in biology, leading to the ridiculous claim that living systems violate the second law of thermodynamics. Another nasty consequence is the idea that the sun is a source of energy. As explained above, energy being by essence a conserved quantity there is neither source of energy nor high-energy molecules in the universe. We have shown above that chemical bonding is the consequence of a confinement that redistributes kinetic and potential energies at constant total energy. Concerning life, we have a low entropy container called the sun pouring high-frequency photons on the earth. But, as energy should always be conserved and entropy should always increase, the earth must in return pour a high number of low frequency photons into the intergalactic space. What have happened in the stars for creating atomic nuclei and in meteorites for creating molecules, also apply to the creation of living cells on earth. Basically, to each reduction of entropy for visible matter corresponds a large increase in entropy carried away by photons. Thus, earth by receiving photons from the sun centered on $\lambda = 0.5$ μm creates photons centered on $\lambda = 10$ μm photons that are emitted towards the intergalactic space. As energy is always conserved, one single photon from the sun (at 0.5 μm) generates 10/0.5 = 20 earth photons (at 10 μm), leaving on earth wonderful and highly sophisticated living struc-

tures. Of course the same 20:1 ratio is retrieved by comparing the temperature of sun's surface computed from Wien displacement law (T = 5760 K) and that of earth surface (T = 288 K = 15°C) as 5760/288 = 20. The fact that we may here use either wavelengths or temperature stems from Noether's theorem stating that energy should always be a conserved quantity (first law of thermodynamics). Speaking of energy consumption or energy sources is thus pure non-sense and biologists should better refer to food (low entropy source, sun) transformed into biomass (low entropy, living species) and heat or waste (high entropy, climate or pollution)[8].

BIOLOGY AND THE SECOND LAW

From the very beginning of its introduction by Rudolf Clausius, entropy was considered as a state function taking definite values for equilibrium states. What was entropy for non-equilibrium states was just ignored as the main focus during the nineteenth century was on optimization of heat engines. Fortunately, thanks to Boltzmann's equation $S = k_B \cdot \mathrm{Ln}\ \Omega$, popularized by Planck and Einstein, we have in hand a generalized definition of entropy applicable to any kind of transformation and that is clearly defined even for non-equilibrium states[43]. Moreover, such a fundamental equation also helps to clarify what lurks behind the notion of an irreversible phenomenon. Let $\Omega_{initial}$ be the phase volume occupied by all microstates compatible with an initial macrostate. In setting up such a state the experimenter's apparatus can put the system only in some uncontrolled point in $\Omega_{initial}$. Then owing to Liouville's theorem stating the conservation of any phase volume by the equations of motion, the process initial → final cannot be reproducible unless the phase volume Ω_{final} is large enough to hold all the microstates that could evolve out of $\Omega_{initial}$. In other words, the requirement that $S_{final} \geq S_{initial}$ (*i.e.* $\Omega_{final} \geq \Omega_{initial}$) is not a mysterious law of nature, but just stems from the need to have a reproducible process[44]. Accordingly, following Boltzmann, Planck and Einstein, any process such that $\Omega_{final} \leq \Omega_{initial}$, should not be considered as forbidden or impossible, but only as improbable; *i.e.*, not reproducible. This is because the ratio of the number of microstates associated to a transformation is given by:

$$\frac{\Omega_{final}}{\Omega_{initial}} = exp\left(\frac{S_{final} - S_{initial}}{k_B}\right)$$

As the smallest entropy difference that could be measured in the laboratory is about 1 $\mu J \cdot K^{-1}$, it follows that for a process such that $\Delta S = S_{final} - S_{initial} = -1\ \mu J \cdot K^{-1}$ one has $\Omega_{final} \approx \Omega_{initial} \times \exp(-10^{17})$. Under such conditions, the final state appears to be so tiny relative to the initial one, that trying to perform the same experiment again and again will always lead to different outcomes. So, it is the mere desire of a human being of studying nature using scientific reproducible experiments that imposes the second law. Fundamentally, anything may happen in nature, but as soon as scientists try focusing on regularities or reproducible facts, then they cannot escape from the second law.

This basically means that perpetual machines of the second kind do exist in nature (we have called them cells) but at the cost of producing non-predictable outcomes (a phenomenon called life). When a scientist pretends that a perpetual motion of the second kind cannot exist, he is right, but then he considers only artificial machines and not living cells. The fundamental keyword characterizing the second law is thus not *disorder* but *reproducibility*. In such a case, it follows that $S = k_B \times \ln\ \Omega$ applies equally well to determining which non-equilibrium states can be reached, reproducibility, from which others and without any restriction to slow, reversible processes. Returning to the case of equilibrium thermodynamics, these considerations lead us to state the conventional second law in the form: *The experimental entropy cannot decrease in a reproducible adiabatic process that starts from a state of complete thermal equilibrium.*[43]

Now, as far as living systems are concerned, the generalization of the second law to non-equilibrium processes appears to be crucial for explaining how the animal muscle succeeds in performing work from activated molecules with 70% efficiency.[45] Accordingly, believing that the muscle behaves as a heat engine, would mean that the maximum attainable work would obey Kelvin's formula for the efficiency $\eta_{max}/\% = 100 \times (1 - T_2/T_1)$ that considers a universal reversible Carnot heat engine operating between upper T_1 and lower temperature T_2. According to this formula, considering a muscle (T_1 = 310K) working at room temperature (T_2 = 300K), one expect that $\eta_{max} = 100 \times (1 - 300/310) = 3\%$! Worst, as soon as room temperature reaches the temperature of the muscle, efficiency drops to exactly zero… To justify the 70% observed efficiency at room temperature, the temperature of the cold reservoir allowing performing mechanical work should be $T_2 = 310 \times (1 - 0.7) = 93K$ = -180 °C. The only correct conclusion to be drawn from these numbers is simply that the animal muscle cannot be a heat engine. But considering the same problem starting directly from Boltzmann's equation and not from Kelvin's one, it transpired that:[45]

$$r = \frac{N_1 \times E_2}{N_2 \times E_1} \Rightarrow \eta_{max}(\%) = 100 \times [1 - r + r \times \ln r]$$

Here, the variable r stands for the non-equilibrium analog of the T_2/T_1 ratio. Being derived under the most general form of the second law, S(initial) ≤ S(final), without restriction of being at equilibrium, this last equation applies to any kind of engine fueled with an energy E_1 focused over N_1 degrees of freedom of the engine and delivered to a large sink reservoir characterized by an average energy $E_2 = ½N_2 \times k_B T_2$. Assuming that energy E_1 is delivered as n quanta of individual energy e = 69 zJ focused on a single vibration mode of the muscle (N_1 = 2n), leads to:

$$r = \frac{½ \times 0.0136 \times 300 \times 2n}{69 \times n} = 0.059 \Rightarrow \eta = 100 \times (1 - 0.059 \times [1 - \ln 0.059]) \approx 77\%$$

Of course, if the quanta of energy were focused on two vibration modes instead of a single one, the maximum efficiency would drop as with N_1 = 4n, we have now r = 0.118, *i.e.* η_{max} = 63%. Had the available chemical energy spread over ten vibration modes before being transferred, the efficiency would be only 10%. The experimental value being 70%, we have here the proof that the muscle is really an amazingly tuned quantum machine and definitively not a heat engine.

Such considerations show how a biological system could be far from equilibrium, even when a thermometer bulb registers a "uniform" temperature within the system. Such a fallacy of thermal equilibrium in a living cell has oriented the whole modern literature of bioenergetics towards Helmholtz's (constant volume) or Gibbs' (constant temperature) "free energies", that apply only when the reaction proceeds so slowly that thermal equilibrium is established at all times. This basically means that heat flows and diffusion fluxes are rapid enough, to maintain uniformity. In a living cell where molecules are not free to diffuse rapidly owing to the presence of membranes (compartmentalization) the best thing to do is thus to rely exclusively on Planck's function, which measures the total entropy discharged in the universe without the constraint of being connected to a thermostat.

With all these clarifications in mind, it should now be clear that non-spontaneous transformations occurring under ambient pressure and characterized by $S_{final} < S_{initial}$ (non equilibrium), $\Delta\psi < 0$ (equilibrium without thermostat) or $\Delta G > 0$ (equilibrium with thermostat) may in fact occur either in a reproducible way ($\Delta S_{univ} \geq 0$) or in a non-reproducible way ($\Delta S_{univ} < 0$). Of course, as far as living systems are concerned, the non-reproducible evolution ($\Delta S_{univ} < 0$) is completely useless for a single isolated cell and is usually encapsulated under different names such as "hazard", "chaos", "chance", "noise", etc. On the other hand, the reproducible evolution ($\Delta S_{univ} \geq 0$) is strongly valorized under other names such as "necessity", "will", "aim", "determinism", etc. But both fundamentally exists in nature and if one switch from the cell level to the species level, ($\Delta S_{univ} < 0$) transformations becomes valorized taking the name of "complexity" or becomes the central dogma of biology "*Omnis cellula e cellula*"[46], stating that the apparition of a single living cell means that a kind of perpetual motion of the second kind called life is initiated that can never be stopped. And as explained just above, a statement such as ($\Delta S_{univ} < 0$) is the insurance that life taken, as a whole, is a fundamental property of the universe that would always find its ways whatever the external conditions. Life could well be a very slow process under unfavorable conditions, but nothing can prevent its manifestation. This would of course be the case if the constraint ($\Delta S_{univ} \geq 0$) were a real law of nature and not just the need of considering exclusively reproducible events. Because adding such a constraint means apparition of an apparent time arrow reflecting the mere fact that macrostates with large multiplicities are, for purely statistical reasons, systematically "favored" over macrostates with low multiplicities.

So, it is somehow satisfying to see that the formalism of thermodynamics leads to the same conclusion as general relativity or quantum mechanics that time fundamentally does not exist. Time is a pertinent attribute only for reproducible processes and if such a constraint is not applied by a conscious being, everything becomes possible and then the mere notion of time evaporates either in nothingness or in endless eternity. Such a conclusion is also coherent with the fact that consciousness should pre-exist to time, space and matter.[47-49]

THERMAL COUPLING AND THE SECOND LAW

Further clarification is also needed for non-spontaneous reproducible processes that are characterized by $S_{final} < S_{initial}$ and $\Delta S_{univ} \geq 0$. This basically means that a local decrease in entropy is tolerated as it is fully compensated by a much bigger increase in the entropy of the whole universe either through generation of heat or by through generation of wastes that could be particles of matter or particles of light (photons). This possibility of releasing entropy either under a material form or

under an immaterial form stems from Sackur-Tetrode's equation underpinning the fact that mass is itself a form of entropy and that entropy is dependent on the total number of particles created that could be indifferently fermions (matter) or bosons (interactions). Of course, for accepting such an idea, it is mandatory to refer to quantum field theories where matter particles may be created or annihilated at will and where each interaction between fermions is interpreted as an exchange of bosons. So, to observe non-spontaneous reproducible processes in nature, one may involve a coupling either with light as evidenced in photosynthesis or with other molecules as evidenced by chemiosmotic processes, such as oxidative phosphorylation.

But before considering such thermodynamic coupling in living systems, one may first consider coupling in heat engines. As exposed above, thermodynamics was first developed to find the maximum theoretical efficiency during the conversion of heat q into useful work w. The idea behind a heat engine is to dispose of a source of heat q_2 that could be extracted from a heat reservoir at the highest possible temperature T_2. If there is available a cold reservoir at temperature $T_1 < T_2$, then this temperature difference may be exploited to obtain work w:

$$w \leq q_2\left(1-\frac{T_1}{T_2}\right), \qquad q_1 \geq q_2\frac{T_1}{T_2} = q(Carnot)$$

As realized by Carnot, the equality holds if and only if the engine is reversible. In the latter case the "wasted energy" q_l(Carnot) is delivered as heat to the reservoir at temperature T_1. The idea is now not to produce work, but rather to deliver the maximum possible heat to that lower temperature reservoir. This is the conversion problem faced in every home, where one has heat from a gas, oil, wood, or coal flame but wants to heat the house in the most efficient way. Here, we are moving from heat engines to heat pumps. The idea is thus to have an ambient heat reservoir (the outside world) at temperature $T_0 < T_1$, and using a perfect Carnot engine to obtain the heat q_l(Carnot) and using the work w available, to drive a heat pump between T_0 and T_1, yielding the additional heat:

$$q_1(pump) = \frac{T_1 \cdot w}{T_1 - T_0}$$

Applying standard thermodynamics, it thus comes that the maximum attainable heat $q_1 = q_l$(Carnot) + q_l(pump) and the heat extracted from the outside reservoir q_0 are such that:[50]

$$q_1 \leq q_2\frac{T_1}{T_2}\cdot\frac{T_2-T_0}{T_1-T_0}, \quad q_0 \leq q_2\frac{T_0}{T_2}\cdot\frac{T_2-T_1}{T_1-T_0} \Rightarrow G =$$

$$\frac{q_1}{q_2} \leq \frac{1-\frac{T_0}{T_2}}{1-\frac{T_0}{T_1}}$$

As before, equality holds if and only if the process is reversible. It is thus easy to see that there is always a net gain (G > 1) as soon as $T_0 < T_1 < T_2$. This also means that heat may flow spontaneously from room temperature T_1 to a higher temperature T_2 because there is simultaneously a compensating heat flow to a lower temperature T_0. In such a case, one may write with $-q_1$, the heat extracted from the room and $-q_2$ the heat delivered to the hotter place ($T_2 > T_1$) that:

$$\frac{(-q_2)}{(-q_1)} \leq \frac{1-\frac{T_0}{T_1}}{1-\frac{T_0}{T_2}}$$

This shows that no spontaneous heat transfer is possible if $T_0 = T_1$, but as soon as $T_0 < T_1$, heat may flow spontaneously from the cold point T_1 to the hot point T_2 because in the same time more heat is transferred to the cold reservoir. One also sees that the lower is T_0, the higher is the amount of heat flowing from T_1 towards T_2, even if $T_1 < T_2$.

This is the basic idea behind any kind of thermodynamic coupling (here with heat engines and pumps) allowing benefiting from a large global entropy flux for inverting locally a smaller entropy flux. Such simple thermodynamic considerations help explain how life apparition on a planet may starts as soon as it becomes cold enough for allowing efficient thermal coupling between hot organisms working at temperature $T_2 \approx 37$ °C drawing heat from a cold surface at $T_1 \approx 15$ °C (greenhouse effect) in thermal contact with a cold huge reservoir at $T_0 \approx$ -18°C (planetary equilibrium temperature). It is worth noting that such a thermal coupling is purely physical and does not depend on the existence of a metabolism based on chemistry. This of course means that warm life is fed by the earth and not really by the sun that behaves as a low entropy source relative to the earth even if it is a high entropy source relative to a icy intergalactic space. It is in this precise sense that life on earth is intimately non-mechanically coupled to what happens at the scale of the whole universe and why thermodynamics is a quite subtle science relative to mechanics or electromagnetism.

CHEMIOSMOTIC COUPLING AND THE SECOND LAW

What can be done with heat may obviously also be realized through chemistry, as atoms and molecules may be considered as "canned heat". Let us assume that we dispose of a chemical reaction able to liberate a given quantity of entropy to the whole universe $\Delta\psi > 0$. One may then consider that Boltzmann's constant k_B could behave as a universal quantum of entropy for evolution, just as Planck's constant h corresponds to a quantum of action for motion. With such a quantum, one may write that $\Delta\psi = N\times k_B > 0$. Let now assume that we want to perform a non-spontaneous but nevertheless reproducible chemical reaction characterized by $\Delta\psi' = -N\times k_B < 0$. The question is how could we may benefit from the fact that N > N'? Let also $\eta = n\times N'/N$ be the efficiency of the coupling. Here we have to consider the fact that we are dealing with basically irreversible processes (chemical reactions) and that part of the entropy has to be necessarily evacuated as heat. This means that the efficiency can neither be $\eta = 1$ (reversible unrealistic case) nor $\eta = 0$ (no coupling at all as all the entropy is exported as heat). The question is thus to find the optimum value for η (or n).

Now, from thermodynamics of irreversible processes we know that not very far from equilibrium, it should exist linear relationships between disequilibrium degrees, D, and corresponding flows, $J = L\times D$[51], where L is a phenomenological coefficient that corresponds to conductance for electrical conduction (Ohm's law $I = \Delta V/R$), diffusion coefficient for diffusion (Fick's law $J_c = -D\times dc/dx$), thermal conductivity for heat conduction (Fourier's law $J_q = -\lambda\times dT/dx$), kinetic constant K for advancement of a chemical reaction (Prigogine's law $J_S = -K\times\Delta\psi$). Focusing on the chemical case, we should have:

$$\frac{J_S}{K\times k_B} = -n\times(N - n\times N') = n^2\times N' - n\times N$$

Derivation of this relation against n, then shows that the optimum efficiency is obtained when n = N/2N' or $\eta = 0.5$. This means that 50% of the available entropy should be used for creating a low entropy mixture (biomass and wastes) and the remaining 50% evacuated as heat. Such a result is perfectly understandable as low values of n means bad coupling, and thus large production of heat. Such a situation is kinetically good because the liberated heat promotes a high disequilibrium degree, giving a large flux of entropy. Conversely, high values of n mean good coupling with low-heat production. But in such a case the disequilibrium degree is low and the kinetics bad, giving a small entropy flux. A good compromise between speediness and efficiency is reached when entropy is equitably shared for creating both matter and heat.

Such considerations allow, on the most general grounds, retrieving clear definitions for different states: life with healthiness ($\eta = 0.5$), life with catabolic illnesses ($\eta < 0.5$) or with anabolic illnesses ($\eta > 0.5$) and of course death by combustion ($\eta = 0$) or death by accumulation of matter ($\eta = 1$).

REFORMING BIOLOGICAL THINKING

It follows from the above analysis that any kind of biological thinking should be centered on the concept of entropy of the whole universe and not on energy. Moreover, the fact that free energies are in fact entropies urges for a reform of the vocabulary. This could be easily done obvious by focusing exclusively on Planck's function, $\psi = S - H/T = -G/T$ that clearly emphasizes its entropic nature while keeping the historical separation between entropic and enthalpic effects. The proposed reform would greatly simplify the subject, as instead of using a counterintuitive $\Delta G \leq 0$ condition for spontaneous evolution at constant temperature and pressure, one would have $\Delta\psi \geq 0$, in straight line with the second law. The term energy would then be reserved for discussing molecular properties where a clear definition as the eigenvalue of a Hamiltonian operator is available. This would have the consequence of rendering facultative the presentation of the so-called "first law", as for macrostates, such principle is more a recipe associated to the definition of the macrostate rather than the expression of a fundamental law of nature. Of course, the law of conservation of energy for microstates would keep its fundamental nature, as it is deep-rooted in Noether's theorem and not linked to the empirical definition of what is a macrostate.

Concerning thermodynamic databases compiling Gibbs' free energies of formation for numerous chemical compounds a simple rescaling, $\Delta\pi_i^\circ = -\Delta_f G^\circ/T$ would be necessary. Here, the symbol $\Delta\pi_i^\circ$ should be understood as an "irreversibility potential" measuring the maximum amount of entropy, hold by a given substance relative to the elements taken in their standard state, that could be irreversibly transferred from the substance to the whole universe during a chemical transformation. The new convention, already used in a previous paper,[8] would then be that for each transformation it exists a thermodynamically allowed spontaneous irreversible direction ($\Delta\pi_i^\circ > 0$) and another direction ($\Delta\pi_i^\circ < 0$) that imperatively needs a coupling with another reaction ($\Delta\pi_i'^\circ > -\Delta\pi_i^\circ$) to have

($\Delta\pi_i$'° + $\Delta\ \pi_i$ °) > 0. Gibbs' free energy of formation from the elements taken in their standard states that are needed for giving numerical values to irreversibility potentials may be derived either indirectly for calorimetric measurements ($\Delta G = \Delta H - T\cdot\Delta S$) or through measurement of redox potentials E ($\Delta G = -n\cdot F\cdot E$, with $F \approx 96\ 500$ C·mol^{-1} and n the number of electrons involved). Many compilations of such values exists in the literature such as NIST-JANAF Thermochemical tables for molecules,[52] U.S. geological survey bulletins for minerals[53] and IUPAC technical reports for radicals.[54]

Concerning units, one should obviously stick to the international practice of expressing energy E in Joules (J) and entropy S in J·K^{-1}. However, one Joule being the energy associated to displacement of a mass m = 1 kg at a speed of v = 1 m·s^{-1} is not very convenient for biology where everything happens with molecules ($m \approx 10^{-27}$ kg) at a nanometer scale ($d \approx 10^{-9}$ m). Fortunately, it exists only six universal constants available for dealing with energy at different scales:

- Einstein's constant (c = 299.792458 µm·s^{-1}) linked to mass m: $E = m\times c^2$.
- Newton's gravitational constant (G = 66.7384 pJ·mkg^{-2}) linked to size R: $E = G\times m^2/R$.
- Planck's constant (h = 662.607015 zJ·fs) linked to frequency f: $E = h\times f$.
- Boltzmann's constant (k_B = 13,80649 yJ·K^{-1}) linked to temperature T: $E = \frac{1}{2}k_BT$.
- Coulomb's constant (e = 160,2176634 zC) linked to electrical potential U: $E = e\times U$.
- Sommerfeld's constant ($\alpha = \mu_0 c\times e^2/2h = 1/137$) linked to electric current I: $E = 2h\times\alpha\times I/e$.

Now, as far as biology is concerned, two obvious qualities emerges U ≈ -100 mV, the membrane potential and T ≈ 310K, the temperature of the human body, leading to E_{pot} = -0,1×160.2 = -16 zJ and E_{temp} = 310×13.81/2 ≈ 2 zJ, with 1 zJ = 10^{-21} J. It thus appears that the zeptojoule (zJ) is a quite convenient unit of energy for quantifying biological processes. This seems to be a much better idea than constantly referring to the energy associated to the irreversible hydrolysis of ATP, which is free energy and thus entropy. This explains why, depending on experimental settings this "reference" value may be anywhere between 35 and 70 zJ depending on the available concentration of magnesium ions.[55] As membrane potential, body temperature and hydrolysis of ATP always amount to a few or at most tens of zeptojoules, such sub-multiple of the joule appears to be a very convenient unit. For chemists and physicists that are not acquainted with such unit, we have the following approximate conversion factors: 1 kJ·mol^{-1} = 1.66 zJ (chemistry) and 1 eV = 160.2 zJ (physics).

It could however happen that the only experimentally available data is the standard enthalpy of formation $\Delta_fH°$. In such a case, one may evaluate entropy of a species of molecular weight M and spin S at temperature T and external pressure P through the following relationship:

$$\frac{S}{k_B} = \frac{5}{2}\ln\frac{T}{T_0} - \ln\frac{P}{P_0} - \frac{3}{2}\ln\frac{M}{M_0} + \Xi + q_{rot} + q_{vib} + \ln(2S+1)$$

Here Ξ is Sackur-Tetrode's constant taking the value Ξ = -1.1517078 for T_0 = 1K, P_0 = 100 kPa and M_0 = 1 Da = 1 g·mol^{-1}, while k_B = 0.01380649 zJ·K^{-1} is Boltzmann's constant. The partition functions q_{rot} and q_{vib} make a zero contribution for mono atomic species. For diatomic species, the entropy will depend on a symmetry number σ = 1 (AB case) or σ = 2 (AA case), and on two spectroscopic constant B_e (rotational constant) and ω_e (vibrational constant):

$$q_{rot}(AB) = 1 + \ln\frac{k_BT}{hcB_e\sigma},\quad x = \frac{hc\omega_e}{k_BT},\quad q_{vib}(AB) = \frac{x}{e^x-1} - \ln(1-e^{-x})$$

If B_e and ω_e are expressed in cm^{-1}, we have = hc/k_B = 1.4388 cm. It is worth nothing that the vibrational contribution is significant at T = 298.15K only if ω_e < 1000 cm^{-1}. For polyatomic molecules containing N atoms, contributions from every vibrational mode (3N – 5 modes for a linear molecule and 3N-6 otherwise) should be added. In such a case, the rotational partition function, depends on the three principal moments of inertia I_1, I_2 and I_3:

$$\theta_n = \frac{h^2}{8\pi^2 k_B I_n} \Rightarrow q_{rot} = \frac{3}{2} + \frac{1}{2}\ln\left(\frac{\pi T^3}{\theta_1\theta_2\theta_3}\right) - \ln\sigma$$

Here the symmetry number is σ = 1 (point-groups: C_1, C_i, C_s or $C_{\infty v}$), σ = 2 (point-group $D_{\infty h}$), σ = n (point-groups: C_n, C_{nv} or C_{nh}), σ = 2n (point-groups: D_n, D_{nh} or D_{nd}), σ = n/2 (point-group S_n), σ = 12 (point-groups: T or T_d), σ = 24 (point-group O_h) or σ = 60 (point-group I_h). Knowing the absolute entropy, it is possible computing an entropy of formation from elements in their standard states $\Delta_fS°$ and the associated irreversibility potential $\pi_i° = \Delta_fS° - \Delta_fH°/T$.

The above considerations apply to species in a gaseous state. For neutral species, the change in irreversibili-

ty potential induced by hydration may be evaluated from Henry's constant $H°_{cp}$ according to:

$$\pi_i^0(X, aq) = \pi_i^0(X, g) + k_B \ln \frac{H_{cp}^0}{H_{ref}}$$

This expression is valid for H_{ref} = 1 M·atm^{-1}, meaning that gas solubility and partial pressure are expressed with units mol·L^{-1} and atmospheres respectively. Henry's constants for numerous gases have been tabulated.[56] For anions and cations, a rough but convenient way of treating hydration is the Born-Mayer equation needing 3 parameters: the electrical charge z, a molecular radius r and the relative dielectric constant of the solvent ε_r:[57]

$$\psi(X^{z\pm}, aq) = \frac{e^2}{8\pi\varepsilon_0} \times \frac{z^2}{r \cdot T} \times \left(1 - \frac{1}{\varepsilon_r}\right)$$

With $e^2/4\pi\varepsilon_0$ = 230.71 zJ·nm, we have for T = 298.15 K and ε_r = 78.4 it comes that $\pi_i° = 0.38197 \times z^2/r(nm)$. For getting more accurate values considering the structure of the water molecules around the ions, one should rely on molecular dynamics simulations.

CONCLUSION

Time should now be ripe enough for replacing the term "bioenergetics" by "biothermodynamics", stressing the fact that energy is a property attached to individual microstates and entropy a property associated to macrostates, *i.e.* to large (typically 10^{24}) collections of microstates (multiplicity Ω). This basically means that entropy is meaningless for individual microstates and that energy is also meaningless for a given macrostate. In fact, speaking of energy is only pertinent when considering a system made of a single unbreakable entity whatever its size that may be atomic (quantum mechanics) or macroscopic (classical mechanics of rigid bodies). In such a case, energy corresponds to the possible eigenvalues of a quantum-mechanical Hamiltonian operator (atoms and molecules) or to the sum of a kinetic contribution proportional to mass times the square of a velocity and of a potential contribution function of the square of spatial coordinates (rigid macroscopic bodies). As soon as one is facing a system made of many similar entities having independent motion, the pertinent variable becomes entropy; energy then being a loose concept whose exact meaning depends on the set of variables controlled by an experimenter for defining a macrostate. This obviously greatly simplifies the presentation of thermodynamics with just a definition of what is entropy, $S = k_B \times \ln \Omega$, and single law of evolution $\Delta S_{univ} \geq 0$. By contrast, the standard presentation sticking to history that uses three different "laws": U = q + w = constant (Kelvin's first law), $\Delta S \geq 0$ (Clausius' second law) and S = 0 if T → 0 (Nernst's theorem or third law) is full of very subtle pitfalls that have been examined with full details in this paper.

Accordingly, using Boltzmann's equation, Nernst's theorem becomes a platitude as by definition $\Omega \geq 1$, with $\Omega \to 1$ when T → 0. Just writing $\Delta S \geq 0$ without referring to the fact that one is considering entropy of the whole universe explains Schrödinger's first error. Finally, adding heat q, which is the product of entropy's flux by a thermal potential, and work, which is the product of a force by its displacement is highly misleading, the only justification being that both quantities share the same physical unit (joules). Thermodynamics is in fact a quite subtle science because it has one foot deep-rooted in quantum mechanics, as the principle $\Delta S_{univ} \geq 0$ is just the expression of Heisenberg's uncertainty principles for a large collection of similar objects. And because one has to consider the whole universe that is the only physical system being really isolated from its surroundings, it has another foot deep-rooted in cosmology through the Bekenstein-Hawking entropy of a black hole characterized by the surface A of its event horizon:

$$\frac{S_{BH}}{A} = \frac{k_B c^3}{4G\hbar} = \pi\alpha \frac{k_B c^2}{G\mu_0 e^2}$$

Such relations show that entropy per unit area is the unique physical concept able to weld all known universal constants (c, G, h, k_B, e, α and μ_0) into just 2 compact scale-invariant quantities. The first relationship emphasizes the material character of the universe (fermions for building structures), while the second one emphasizes its complementary immaterial character (bosons for transmitting forces stabilizing structures). The intimate link between entropy and time suggested by the $\Delta S_{univ} \geq 0$ constraint for reproducibility is further indication that life speaks the language of entropy (or its immaterial version, information) and not that of energy. The domain where such reformulation will bring about conceptual breakthroughs is obviously medicine as already suggested[58] and further developed in forthcoming papers.

After reviewing of these ideas by anonymous referees, several comments need to be added to this conclusion. Stressing that biology and medicine are currently on a wrong way does not mean that thermodynamics, quantum mechanics and chemistry are free of defaults. If there are no doubts that life relies extensively on far from equilibrium thermodynamics, one may argue that

such thinking apply also to abiotic systems. This implies that living systems are in some way at another level of thermodynamics of irreversible systems. But, it is worth recalling that irreversibility may be considered by two different theories. There is the linear theory, extensively developed by Brussels' School, and the non-linear theory needed to describe chaotic systems. Again, there are little doubts that the non-linear theory of chaos should be the right way of thinking for a good understanding of living systems. This simply stems from the fact that the linear thinking is just a special case of the non-linear one. But climbing at the non-linear level is no guarantee that we are at the top. Because, an essential ingredient of life is still missing: consciousness. I will not go further here because the interplay between consciousness and life has been extensively discussed in previous papers [2, 59, 60]. This basically means that cleaning up the mess at the nuts and bolts level is also needed in quantum mechanics and chemistry.

This last point was pinpointed by one of the referee and if not properly discussed, it may seem that by focusing on biology and biology, I am putting the cart before the horse. I fully agree with this view, stating that there absolutely no guarantee that quantum physics, lying behind entropy, is not badly flawed. Accordingly, we know that the entire mystique surrounding quantum physics could be easily avoided. Thus, to justify Planck's blackbody spectrum, the entry-point of quanta in physics, we just need: the equivalence principle, the assumed absence of a perpetual motion machine in a classical gravitational field and classical electromagnetic zero-point radiation (see [61] and references herein for more details). It is worth stressing that in this no-quantum we absolutely need absence of a perpetual motion machine. This basically means that we absolutely don't need the quantum mystique for stressing the crucial role of entropy. If I have chosen here to favor a quantum flavor of physics, this is just because quantum physics belongs to the current paradigm. But, relying on quantum principles is definitively not a prerequisite for an entropy-based reformulation of biological thinking.

One should also be aware that chemistry was at the end of nineteenth century a powerful horse for thinking "quantum". I have even defended elsewhere the idea that chemistry is in fact irreducible to quantum physics.[62] And if this is true, it then logically follows that biology should also be irreducible to quantum physics. This stems from the fact that both sciences rely extensively on thermodynamics. There is now a convergence towards the idea that scaling symmetry is the missing ingredient of contemporary physics,[63,64] chemistry[65] and biology.[60] The only needed discussion is how entropy deals with scaling symmetry. It is at this point that enters information theory as explained elsewhere.[60] It should be however crystal clear that this does not imply that computers should be the next stage of progress, as computers are only able manipulating information that is devoid of meaning. By contrast, living systems can manipulate entropy fluxes to create information full of meaning. Again, this is because consciousness lies above information, entropy or matter.[59] Computers should then be viewed as mere technical and stupid tools for conscious beings and not as intermediates in the emergence of consciousness from matter. In such a new paradigm, there is even a place for the role of dissolved gases in water. This stems from the fact that information processing in living systems is based on water and not on silicon. This is precisely why there is so much water in any living cell. And water without dissolved gas cannot hold the information long enough to be processed. Obviously, water with gases should no more be called water. It should be called interfacial,[66] zoemorphic,[67] morphogenic,[68,69] EZ-water[70] or what you want but please don't call it "water".

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Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Research Articles

Darwin and Inequality

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Abstract. Charles Darwin during his travels on the Beagle noted the wretched primitive state of natives from Tierra del Fuego. He attributed it to their being egalitarians and to the absence of a leader among them. The Gini Economic Index suggests strong inequality today even among the richest, more developed countries. This paper discusses whether equality and civilization are really incompatible, as Darwin seemed to imply.

Keywords: Inequality, Darwin, Gini Index, Egalitarian Societies.

OPEN ACCESS

Citation: Bonatti E. (2021) Darwin and Inequality. *Substantia* 5(1) : 73-78. doi: 10.36253/Substantia-1121

Received: Sep 27, 2020

Revised: Nov 22, 2020

Just Accepted Online: Nov 23, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Charles Darwin during his 1831-1836 circumnavigation of the Globe on the British Navy brig "Beagle" had a chance to interact with the native inhabitants of the eastern and northern shores of Tierra del Fuego (Figure 1).

Darwin dedicated several pages of his "The Voyage of the Beagle" to the Fuegians, and already from the first encounter with the natives he expressed his amazement at their wretched primitive state (Figure 2). Here are a few citations: *"I could not have believed how wide was the difference between savage and civilized man…it is greater than between a wild and a domesticated animal…"*… *"While going one day on shore near Wollaston Island we pulled along side a canoe with six Fuegians. They were the most abject and miserable creatures I anywhere beheld …These Fuegians in the canoe were quite naked, and even one full grown woman was absolutely so. It was raining heavily and the fresh water together with the spray, trickled down her body.…In another harbour not far distant a woman who was suckling a recently born child, came alongside the vessel and remained there while the sleet fell and thawed on her naked bosom and on the skin of her naked baby…these poor wretches were stunted in their growth, their hideous faces bedaubed with white paint, their skins filthy and greasy.…"…Viewing such men, one can hardly make oneself believe that they are fellow creatures and inhabitants of the same world.…"*

Later on in his narrative Darwin offers an explanation of the wretched state of the Fuegians: "…… *the perfect equality among the individuals composing the Fuegian tribes must for a long time retard their civilization. As we see, those animals, whose instinct compels them to live in society and obey a Chief, are most capable of improvement, so is it with the races of humankind. Whether we look at it as a cause or a consequence, the more civilized*

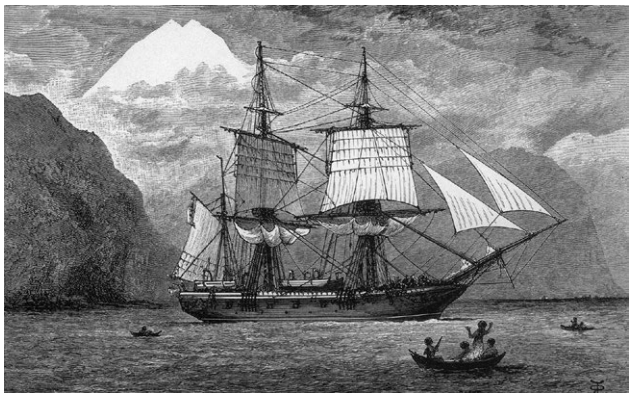

Figure 1. The Beagle in The Strait of Magellan, 1833. (from The Voyage of the Beagle, edition 1962, American Museum of Natural History).

always have the most artificial governments. For instance, the inhabitants of Tahiti, who when first discovered were governed by hereditary kings, had arrived at a far higher grade (of civilization) than another branch of the same people, the New Zealanders, - who, although benefited by being compelled to turn their attention to agriculture, were republicans in the most absolute sense. In Tierra del Fuego, until some Chief shall arise with power sufficient to secure any acquired advantage, such as ownership of domesticated animals, it seems scarcely possible that the political state of the country can be improved. At present even a piece of cloth given to one is torn into shreds and distributed; and no one individual becomes richer than another. On the other hand, it is difficult to understand how a Chief can arise till there is property of some sort by which he might manifest his superiority and increase his power." (Darwin, 1962 edition, pages 205-231).

That Darwin would hold this opinion is quite understandable: after all, he was a member of the pre-Victorian England's higher classes. Still, we ask: ARE *EQUALITY AND CIVILIZATION REALLY INCOMPATIBLE?*

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The issue of inequality among individuals, social classes and nations has been debated widely in recent years (see Chien and Culotta, 2014). Economists use a "Gini Index" (Gini, 1912) to measure the level of inequality among the individuals of a group or a country. The Gini Index is a measure of the distribution of income across a population. It was devised early last century by Corrado Gini (1883-1965), an Italian statistician and economist who thought at the University of Roma. The Gini Index ranges from zero if all individuals of the group own and earn the same amount; to one if one individual owns and earns everything and everybody else owns and earns nothing. The Gini index estimated for various countries reveals strong variations in degree of inequality (Figure 3). We go from a relative equality in the Scandinavian countries (Norway and Sweden have a Gini index around 0.25) to a strong inequality in countries such as South Africa (index close to 0.7). European countries such as Great Britain; France, Germany and Italy have indices ranging between 0.28 and 0.35 while the US suffers from a rather high inequality (index ~ 0.45), as do China and Russia.

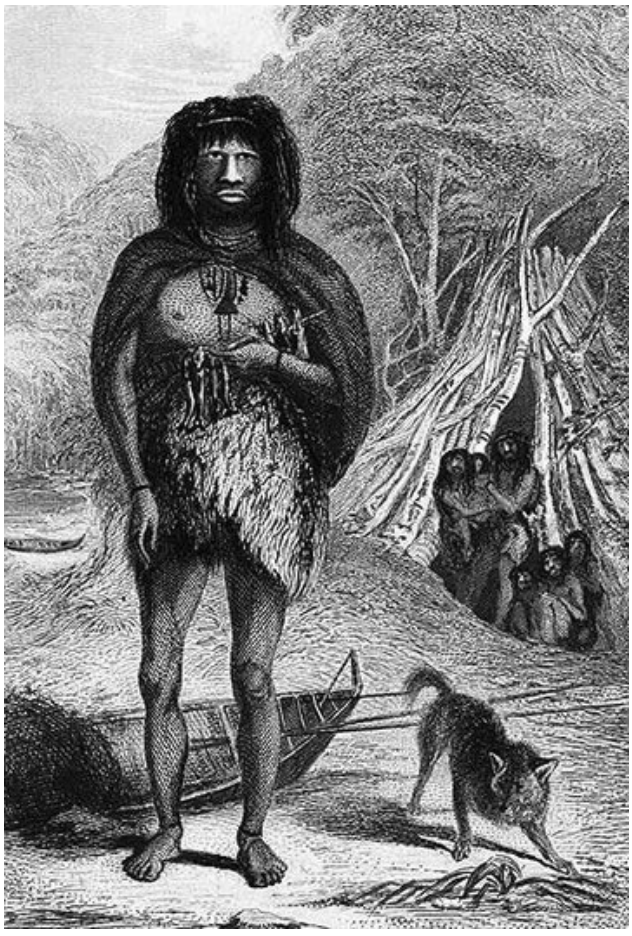

Figure 2. Native from Tierra del Fuego, drawn by Conrad Martens, artist on board of the Beagle, 1833.

Figure 3 suggests that some of the richest countries on Earth suffer from high degrees of inequality. High levels of inequality (i.e., high Gini Indices) generally go together with a number of negative indices: for instance, they correlate with the percentage of children living in poverty (Atkinson, 2015) as shown by Figure 4. Inequality in a society predicts a higher degree of violence (Wilkinson and Pickett, 2009; Starness et al., 2017).

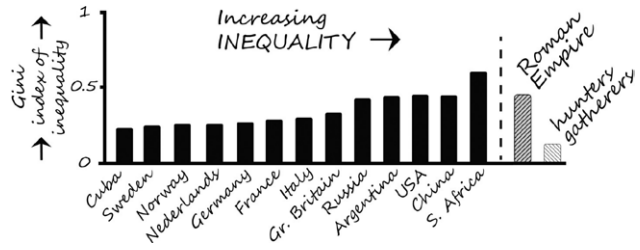

Figure 3. Gini Index of Inequality estimated recently (within the last 10 years) for various countries. The index for Cuba refers to the period 1980-1985. Scandinavian countries are the most egalitarian. Russia, US and China show a much higher "inequality". The index reconstructed for the Roman Empire (Sheidel and Friesen, 2009) reveals an inequality comparable to that of modern US. Also shown is the Index for pre-agriculture hunters-gatherers estimated by Kohler et al. (2017).

Areas in the US with high income inequality tend to have higher divorce, bankruptcy and homicide rates than areas with more egalitarian distribution (Frank et al.,2014; Daly et al., 2001). In the United States people in the low-income bracket are plagued by mortality risk and rates of infant mortality higher than the general population (Underwood, 2014).

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Are there in today's Earth societies where inequality is negligible? Shostak (1981) and Pennisi (2014) describe the !Kung people of the Kalahary Desert in Africa. They are nomadic hunter-gatherers; they share their very few possessions (mostly food and hunting weapons): so nobody is "richer" than anybody else. Another group of nomadic hunter-gatherers are the Hadza people who live at the margins of the Rift Valley in Tanzania (Gibbons, 2018). They have been studied by several teams of anthropologists for over 50 years. They also were egalitarians; however, their way of life has been heavily infringed in the last several years by the encroachment of farmers and pastoralists but also of tourists and of the same anthropologists who studied them (Gibbons, 2018).

Before the invention of agriculture over 10,000 years ago, humans were mostly nomadic "hunter gatherers"; anthropologists believe their societies were generally rather egalitarian, as are today's !Kung. The average Gini index for pre-agriculture "hunter-gatherers groups" has been estimated at 0.17; that for agricultural societies at 0.35 (Kohler et al., 2017). In his book "The Anatomy of Inequality" (2016), the Swedish social philosopher Per Molander suggests that the prevalence of egalitarianism among nomadic "hunter-gatherers" is mostly a consequence of their economy being close to subsistence level:

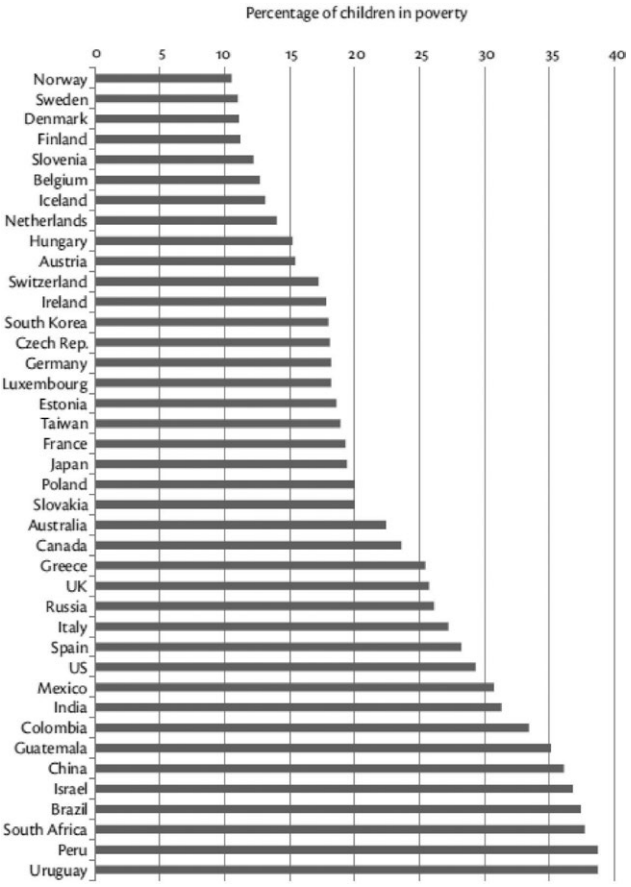

Figure 4. Percentage of children (persons < 18 years old) living in poverty from various countries in 2010 (from Inequality: what can be done? By Anthony Atkinson, Cambridge, Mass.: Harvard University Press, Copyright © 2015 by the President and Fellows of Harvard College). Note that countries with high index of inequality (see Fig.3) tend to have a high percentage of children living in poverty.

no significant surplus is there to be grabbed by an individual or group. However, archaeologists are finding traces of early inequality already in some "hunter-gatherers" groups, where a few individuals were able to take possession of patches of wild food and transmit them to their descendant, in what can be viewed as *the BIRTH OF PRIVATE PROPERTY.*

For instance, Price and Bar-Joseph (2000) found archaeological traces of inequality already in Natufian tribes, eastern Mediterranean hunter-gatherers that from 14,000 to 10,000 years ago gradually were converted to agriculture. The richness of a few of their graves and the ornaments on a few of their dead indicate that disparities existed early on, before the Natufians settled down in a society based on agriculture. Accumulation of wealth by a few, and consequent inequality, became

"normal" in societies based on agriculture. Molander (2014) cites 4,000 years old Sumerian and Egyptian texts that lament inequality in their society.

The transition from nomadic hunter/gatherers to stable societies based on agriculture has been regarded generally as a major positive step in the evolution of humans towards higher civilization. A dissenting viewpoint, voiced by Jared Diamond (1987) in an essay entitled "The Worst Mistake in the History of the Human Race", argued that the advantages derived from this transition were more than balanced by negative effects. Diamond cited studies on the few remaining hunter/gatherers communities, as well as on fossil and archeological records of ancient communities, showing that the "quality of life ", including nutrition and health, did not necessarily improuve as a result of the transition, except for a small elite group. With the advent of agriculture population density increased: quality was traded for quantity….an elite became better off but most people became worse off, with the result of developing deep class divisions. Descriptions of nomadic egalitarian societies such as the !Kung of southwest Africa (Shostak, 1981) and archaelogical records from neolithic sites (Patou-Mathis, 2020), suggest that women were freer and less unequal than in later societies based on agriculture and private property.

According to Canadian archaeologist Brian Hajdess, the transition from egalitarian societies to societies rife with economic competition and inequality was *"the single most critical watershed in the 2.5 million years of human history"*. Since that watershed transition, stratified societies have prevailed among humans, although the extent and type of inequality changed in time and space and was different in different societies. In Europe and the Middle East we had strong inequality in the Persian and Egyptian Empires and even in the 400 BC quasi-democracies of Athens and Sparta, that allowed slavery. We had a strongly unequal society also in the Roman Imperial period. A Gini index of 43 was estimated for imperial Rome (Sheidel and Friesen, 2009), with a degree of inequality similar to that of today's US. A few super rich Romans thrived: triumvir Marcus Crassus had an income roughly equivalent to one billion dollars per year, not far from that of Bill Gates!

Non-egalitarian societies continued to prevail in the European Middle Ages and Renaissance, sometime favoured by "holy" (or, better, "unholy") alliances between religious (The Pope) and lay (The Emperor) leaders. Throughout Humanism, The French Revolution, The Industrial Revolution, and the rise of the bourgeoisie, the degree of inequality oscillated but overall did not decrease. A concrete modern attempt to create something approaching an egalitarian society, i.e., the 1917 Russian revolution, ended badly due both to internal failures and to external pressure from capitalistic powers. Scholars such as A. Bergson (1984) have attempted to estimate the degree of equality in the Soviet Union before and after the death of Stalin. The results of these inquiries are ambiguous, in part because it is not simple to calculate a Gini Index for a Soviet Union-type economy. Even so, it appears the Soviet Union had a slightly higher equality than western countries. Cuba in the early eighties enjoyed a relatively high equality (Gini index 22 to 24), that however decreased more recently. It's worth noting that the Gini index of modern post-socialist countries such as Russia and China is not very different from that of the foremost capitalistic country, i.e., the US (Figure 3). Chairman Mao would turn in his grave if he knew that many billionaires sit in today's Chinese Parliament (NY Times, March 17, 2017).

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Given that we are all born with different intelligence, physical strength and so on, and that most of us want the best for ourselves even at the expense of others, Pennisi (2014) asks: How has it been possible for egalitarian societies to survive among our ancestors? A widespread opinion is that inequality is an inescapable consequence of human nature: *Homo sapiens* basic instincts lead him/her to be competitive and possessive. How to reconcile this hypothesis with the evidence that pre-agriculture hunter-gatherers societies were mostly egalitarian? Studies of the few remaining hunter-gatherers egalitarian groups (they are disappearing fast!) hint at some sort of "non-aggressive" code promulgated in those societies: for instance in the !Kung (Shostak, 1981) and Hazda (Woodburn, 1983) people. Behaviour fostering inequality (boasting, self-aggrandizing, competitiveness) is discouraged even in young children while humility, downplaying one's accomplishments and cooperation (for instance in hunting) were the accepted ways of behaviour. It is interesting that in most aspects of our western societies, for instance our school systems, exactly the opposite behaviour is encouraged. Then perhaps the drive towards inequality may not be due solely to our genes or to "human nature", but rather to which aspect of human nature is being reinforced and encouraged, and which aspect neglected and discouraged. The degree of inequality has varied through time: for instance, it decreased in European countries during the years of the second world war but increased in the post-1970

period (Atkinson, 2015). It has varied from place to place depending on the social organization of each country. Moreover, small communities exist with little or no inequality (Israeli kibutz, monasteries of various religions…). All this supports the idea that inequality is not an inescapable consequence of human nature.

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French economist Thomas Piketty suggested that, once even a small inequality has surfaced in a society (that is, once an individual or group have acquired wealth and power slightly over and above the rest of society), inequality is bound to increase with time. The simple reason, hinted at not only by mathematical models but also by common sense, is that in a competitive society who starts with more is likely to win the competition to acquire more. Modern "liberal" societies have introduced devices to avoid being overwhelmed by extreme inequality. *Constitutions* proclaim equal dignity and rights for all individuals. *Graded taxation* requires the richest to pay higher taxes. *Social security* and *healthcare* attempt to keep the weakest protected. *Tax on inheritance* and *universal education* attempt to avoid excessive accumulation of wealth and to approach equal opportunity for youngsters. On this last point: a strong correlation between income of parents versus income of their children (calculated when the children have become adults) indicates a low social mobility and a low degree of "equal opportunity". Italy tops this ladder (Figure 5), followed closely by Great Britain and US, a sign of a relatively low "equal opportunity" for youngsters from those countries. Canadian economist M, Corak (2013) found that countries with high correlation between parents/children income tend to have a high Gini index of inequality (Figure 5).

According to Piketty, we are facing a sharp rise in inequality in the capitalist west. In fact, the trend in recent years in the western nations, particularly the US, seems to move even further away from egalitarianism: for instance, if it is true that new tax laws will shift wealth to the wealthiest (New York Times), the US Gini Index would be pushed even further up. In recent years, some of the guarantees against excessive inequality have been slackened in various countries of the west. Even "virtuous" Sweden has abolished its tax on inheritance and has seen its index of inequality go up in the last several years (Molander, 2016). On the other hand, studies summarized by Starmans et al. (2017) suggest that people are bothered non so much by economic inequality as by economic unfairness. They argue that people favour "fair distribution" over "equal distribution", and prefer

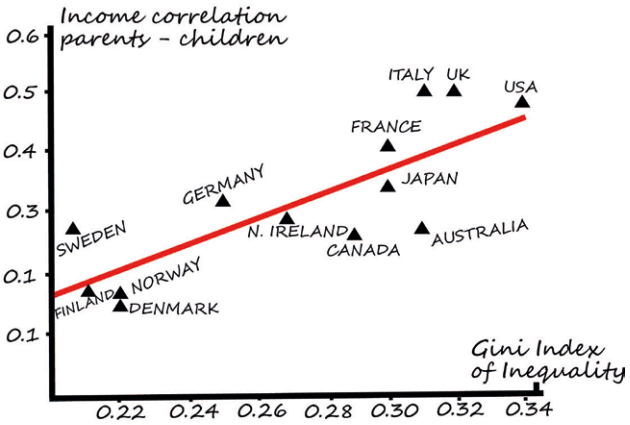

Figure 5. Gini index of Inequality for various countries versus Parents/Children Income. Correlation estimated after children have become adults (from Corak, 2013).

"fair inequality" over "unfair equality". However, much depends on the definition of what is "fair", that varies in different places and in different social classes.

In a review published by the New Yorker (March 31, 2014) of Piketty book "Capital in The Twenty-First Century", J. Cassidy cites extreme examples of inequality in US corporations. The Chief Executive of Walmart Corporation earned more than 23 million dollars in 2012, while a typical Walmart worker earned less than 25,000 dollars a year. The Chief executive of Apple earned 378 million dollars in 2011, about 6,250 times more than the average Apple employee. The British organization Oxfan reports that the 85 richest people in the world own more wealth than the 3,5 billion people who make up the poorest half of world's population!

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Let's go back to Darwin. Let's imagine him sailing today on a modern cruise ship back to Tierra del Fuego: he would see the descendants of his ancient Fuegians, having abandoned their egalitarianism, working dutifully as janitors and dishwashers in the basements of Ushuaia luxury hotels owned by US or Argentinian tycoons. Perhaps Darwin would ask himself whether or not this can be construed as "progress".

ACKNOWLEDGEMENTS

I am grateful to Vincenzo Balzani, Luigi Bonatti, Anna Cataldi, Gino Fontana, Luca Gasperini and two anonymous reviewers for helping improve the quality of this paper.

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Substantia
An International Journal of the History of Chemistry

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Research Articles

Loren Eiseley's Substitution

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Abstract. The anthropologist and acclaimed essayist, Loren Eiseley, in the midst of recounting a vision in the conclusion of a draft of a 1960 composition, "Creativity and Modern Science," invoked Charles Darwin as the essay's animating spirit. Eiseley modified his draft the next year and published it in no less than three of his subsequent books. The most striking differences between his draft and published texts is the substitution of Darwin in the final moments of the narrative with Francis Bacon, a barrister and philosopher who died nearly two centuries before the famous biologist was born. Here, is crafted a rationale for this unlikely switch, to the extent that the intent of another can be uncovered, by closely reading Eiseley's psychologically charged work. Eiseley's own struggles as both a scientist and an artist, identities respectively epitomized by Darwin and Bacon, reveal how and why the writer permitted his foremost heroes to be substituted, one for the other.

Keywords: Francis Bacon, Charles Darwin, Loren Eiseley, Rachel Carson, Richard Nixon.

OPEN ACCESS

Citation: Kahr B. (2021) Loren Eiseley's Substitution. *Substantia* 5(1) : 79-89. doi: 10.36253/Substantia-1040

Received: Jul 31, 2020

Revised: Oct 23, 2020

Just Accepted Online: Oct 26, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

INTRODUCTION: EISELEY AND RACHEL CARSON

"If the world were lit solely by lightning flashes how much more we would see?" is the leading question in an 11-page typewritten essay called "Creativity and Modern Science" (hereafter CMS) by Loren Eiseley (1907-1977) dated 19 September 1960. Less light, see more? A compelling provocation. Here, I compare Eiseley's unpublished draft with the form of CMS in an autobiographical essay collection and two books about the philosopher of science and Lord Chancellor of England, Sir Francis Bacon (1561-1626).[1] My remarks confront the fidelity of ideas in the written word.

Eiseley's text is stored in the Rachel Carson (1907-1964) archive at Yale University.[2] Eiseley and Carson, born the same year, were arguably the most popular so-called Nature writers of their time.[3] Eiseley's break-out book, *The Immense Journey* (1957),[4] about human evolution, has been translated into at least a dozen languages. At the time of its publication, Carson already earned a wide following with her trilogy about the sea.[5,6,7] After reading *The Immense Journey*, she wrote to Eiseley to express admiration for his "magical passages."[8] Eiseley, in turn, reviewed Carson's landmark book, *Silent Spring* (1962),[9] about the misuse of insecticides. Echoing Carson, Eiseley wrote that

many pests once thought to have been eliminated may return "to flourish in a sack of DDT and thus be twice as formidable… [We are] whetting the cutting edge of natural selection but its edge is turned against [us]..."[10]

Concern over the abuse of science and technology were already resonant with Eiseley. As a Cold War-era author, he trained one eye on the sky, ever wary of the missiles that might fall out of it. Even Eiseley's meditations on his favorite subject, evolution, often foresaw an anthropogenic end of the process from which humans arise; isles where castaways could blossom under natural selection are being "flattened into the long runways of the bombers."[11] "We have lived to see", wrote Eiseley in CMS, "the technical progress that was hailed in one age as the savior of man become the horror of the next." Bacon was likewise fearful, in his time before the bombers, recognizing that what we now call *sciences* have "an ambiguous or double use, and serve as well to promote as to prevent mischief and destruction, so that their virtue almost destroys or unwinds itself."[12]

Eiseley grew up in Lincoln, Nebraska. It took him eight years during the Great Depression to earn a bachelor's degree from the University of Nebraska. He spent some of that time riding the rails, recovering from tuberculosis, and collecting bones of huge mammals that once roamed the Badlands. Meanwhile, Eiseley searched for artifacts of ice-age humans, an occupation that strengthened his interest in the developing field of anthropology, the subject in which he ultimately earned a PhD from the University of Pennsylvania. All the while, he wrote poetry and short stories.

Eiseley became an assistant professor at the University of Kansas, moved to Oberlin College as department chair, and then returned to the University of Pennsylvania. When he wrote CMS, he was provost at Penn, an administrative position to which he, his friends, and his colleagues thought him ill-suited.[8]

Eiseley is not remembered best for his scientific discoveries, but rather for his books about natural history. He has been the subject of several biographies,[8,13,14,15] and volumes of critical analysis.[16,17,18] Christianson's comprehensive biography is especially recommended,[8] as is Eiseley's autobiography.[19]

DISCUSSION

Creativity and Modern Science

In September, 1960, Eiseley presumably delivered an address based on the text of CMS, an essay that begins with a lightning strike and the inspiration that comes from a flash, whether a real bolt in a field at dusk during a storm or the metaphorical flash that strikes the creative mind. CMS is about many things: the conformity of thought in modern society, the authoritarianism of science and its abuse by the state in search of power, and the decline of the personal essay, Eiseley's chosen artform that he considered unsurpassed in its ability to express the unique cast of an individual mind. Regarding this last theme, Eiseley invoked the great English naturalists, among them Gilbert White (1720-1793), admired by the young Darwin:[20] "Even though they were not discoverers," remarked Eiseley, "…one feels at times the great nature essayists had more individual perception than their scientific contemporaries."[2] Eiseley aspired to be in White's company, and he succeeded by all accounts.

CMS, characterized as "one of Eiseley's most vivid examinations of the limits of humanism and the limits of science,"[18] closes with a terrifying recollection and a reappearance of the lightning motif from the start of the composition. As a young man lost in a "rural and obscure corner of the United States", Eiseley was collecting insects, including "beetles with armored excrescences". The "inbred" and "misfit" people of the unnamed region were like bugs themselves, evolved to their "odd niche." Eiseley, a loner, wasn't out to befriend these people. He enjoyed solitary work. One day, the sky suddenly darkened with rain. In an instant, he was overwhelmed with "one of those flame-lit revelations which destroy the natural world forever and replace it with some searing inner vision which accompanies us to the end of our lives." In front of him was a "man high on a great load of hay" pulled by a team of horses thundering down the road. Anticipating a rescue from the storm, Eiseley stepped forward to ask for a lift.

> Then, in a bolt of light that lit the man on the hayrick… I had seen a human face of so incredible a nature as still to amaze and mystify me....It was – and this is no exaggeration – two faces welded vertically together along the midline…One side was lumpish with swollen and inexpressibly malign excresences [the same as the beetles he was collecting]; the other shone in the blue light, pale, ethereal, remote – a face marked by suffering yet serene and alien to that visage with which it shared this dreadful mortal frame...I saw the double face of mankind in that instant of vision…I saw man – all of us – galloping through a torrential landscape, diseased and fungoid, with that pale half-visage of nobility and despair dwarfed but serene upon a twofold countenance.

Eiseley saw this but imagined it at the same time. The drama of the lightning impressed upon Eiseley the realization that his mind was transformed what he saw so as to amaze and mystify.

> [I]t is through our minds alone that man passes like that swaying furious rider on the hayrick, farther and more desperately into the night. He is galloping – *this two-fold creature whom even Darwin glimpsed* – across the storm-filled heath of time, from the dark world of the natural toward some dawn he seeks beyond the horizon. [Emphasis added].

Eiseley, forever haunted by this image of a terrified/serene creature uncontrollably galloping through the night, was striking in part because of its haunting intimacy. It recalled Jim Morrison's (1943-1971) ominous last recording which incorporates the sounds of thunder and rain into the vocal track: "Riders on the storm; Into this house we're born; Into this world we're thrown…If you give a man a ride..."[21]

The B-side of *Riders on the Storm* is called "Changeling", a favorite Eiseley word so often used it was like a tic. "I was loved," according to Eiseley, "but I was also a changeling."[19] The last sentences of CMS are these:

> Across that midnight landscape he rides with his toppling burden of despair and hope, bearing with him the beast's face and the dream, but unable to cast off either or to believe in either. For he is man, the changeling, in whom the sense of goodness has not perished, nor an eye for some supernatural guidepost in the night.

The similarities between Eiseley and Morrison – though no direct connection is implied as Eiseley surely eschewed rock-n'-roll – did not inspire my sustained listening to Morrison's band, *The Doors*. Instead, I chose to read what else Eiseley wrote (he wrote a lot but not too much for a quarantine). CMS is strong, and I was motivated to make better sense of it, a guidepost in the night that I stumbled on by chance in the archives of another naturalist.

Eiseley's wrote CMS only months before the calendar turned to a new year, 1961, the four-hundredth anniversary of the birth of Francis Bacon. Eiseley was asked to lecture on Bacon's role as an educator in January at the University of Pennsylvania. The same speech was reprised shortly thereafter at the University of Nebraska. The latter presentation was accompanied by a $1,500 stipend and the promise of a publication based on the oral remarks.[8] However, Eiseley's manuscript, organized in two parts, was disappointingly slender when received by the University of Nebraska Press. The editors requested a third chapter. There followed delays and acrimonious correspondence.[8] Three chapters were ultimately published as *Francis Bacon and the Modern Dilemma* (1962, hereafter *FBMD*).[22] CMS, it turns out, is the second half of the third chapter *FBMD*. The first two chapters had been previously published in the *Saturday Evening Post*[23] and in *Science*,[24] respectively. Like chapter three, they were not original to *FBMD* but drawn from elsewhere. Regrouping existing pieces was a common tactic for Eiseley.

In 1971, the CMS lecture was published again in an essay collection, *The Night Country*.[1] Two years later, Eiseley added a leading biographical chapter about Bacon and published the enlarged work as *The Man Who Saw Through Time,* still containing CMS as it appeared in the earlier books.[25] In all, Eiseley published CMS in some form thrice. For him, it would appear to have been an important composition. This may be because his vision in CMS is an echo of a childhood memory, also published in *The Night Country*.[1] As a boy, Eiseley hopped on the horse-drawn wagon of a merchant who rode through the countryside under "the kind of eternal light which exists only in the minds of the very young." The wagon stopped at the big house of the Bishop of Lincoln which sat behind a black iron fence. Eiseley got off as a storm was approaching: "The thunder from the clouds mingled with the hollow rolling of the wheels and the crash of the closing gates before me echoed through my frightened head with a kind of dreadful finality." The CMS vision revisits these images and feelings. It is deep Eiseley history that he held tightly throughout his life.

Eiseley, Francis Bacon and Charles Darwin

CMS can be justified in a book about Bacon because it discusses the process of modern science, something Bacon is widely credited with having invented. However, except for a passing reference to a "Baconian Utopia," CMS has nothing in particular to do with Bacon. In order to incorporate CMS into a pair of books about Francis Bacon, Eiseley made a small, yet vexing substitution to the penultimate sentence, which I repeat here with the swap emphasized by a ~~strikethrough~~ and **bold** text:

> [I]t is through our minds alone that man passes like that swaying furious rider on the hayrick, farther and more desperately into the night. He is galloping – this two-fold creature whom even ~~Darwin~~ **Bacon** glimpsed – across the storm-filled heath of time, from the dark world of the natural toward some dawn he seeks beyond the horizon.

After linking Darwin in CMS to his youthful, lightning-sparked insight about the human condition – hope and despair joined in a fraught search for something in the night – Darwin is unceremoniously unhitched from the wagon. He is replaced by Bacon in a part of the

argument so urgent and so vivid that such a casual substitution of one giant for another from a different century would have seemed impossible for an author of such precision as Eiseley.

Eiseley referred to his highly autobiographical writing as the art of the "concealed essay" whereby the personal is delivered under cover of some apparently objective disquisition on science or natural history, an integration of autobiographical material, scientific fact, and literary/historical allusions. This switch of Darwin for Bacon turned the personal essay, which Eiseley championed as a high form of human expression, into something uncharacteristically modular.

CMS rails against the prefabrication of American life of the 1950s, "the monotony of our great shopping centers" and "our mass-produced entertainment" where one size fits all or does not really fit anyone but will have to suffice. And yet, Eiseley forced Darwin and Bacon to shop at the same stores and watch the same movies. This switch of two of the most famous contributors to the program of modern science seemed to me to be a clue about something larger, something worth identifying.

In CMS, as originally drafted, Eiseley, collecting beetles while the sky darkens, strongly identified with Darwin; it is widely known that beetles were Darwin's lifelong passion.[26,27] When young Charles found more specimens than he could hold, he famously popped one in his mouth. "[N]o pursuit at Cambridge," Darwin wrote, "was followed with nearly so much eagerness or gave me so much pleasure as collecting beetles."[19] Later, beetle-mania followed whenever the *Beagle* was docked in a new harbor. Among Darwin's last scientific contributions was a description of a clam that had attached itself to a leg of beetle[28] (a specimen which he received in the mail from the grandfather of Francis Crick!).[29] Darwin saw this presumably unwelcome clamping as a mechanism for the biogeographical dispersion of species from their location of evolutionary origin. In CMS, Eiseley was doing the quintessential work of Darwin as the rider approaches in the storm. Eiseley put himself in Darwin's shoes as a fellow beetle collector.

How could Eiseley so easily slip on Bacon's shoes? In the original chapters of *FBMD*, "Bacon" is mentioned three times per page, but the part of the closing chapter corresponding to CMS drops "Bacon" just once every three pages. Eiseley, according to his biographer Christiansen, rarely let a piece of writing go unpublished. He never allowed his lectures to be audiotaped and never distributed texts of oral remarks because everything was "headed for the printer."[8] The third chapter containing CMS was solicited after the fact, and its completion was strained. The switch of Darwin for Bacon appears to be an effort to shoehorn an existing essay into a work, *FBMD*, commissioned to celebrate Bacon, that needed to be fattened to satisfy Eiseley's publishers.

Not surprisingly, some cobbling was necessary to make Bacon fit. Eiseley sprinkled in "Bacon" four times in an effort to mitigate the apparent inappropriateness of the CMS insertion in *FBMD*. For instance, a sentence of CMS is changed in *FBMD* with the addition of the underlined phrase: "We forget – as Bacon did not forget – that there is a natural history of souls..." This comes across as clumsy to any reader attuned to the problem that Eiseley was working out, the "Baconification" of CMS.

Clumsiness spotted in hindsight should not detract from Eiseley's great skill. He was not so careless as to let a chapter of newly published prose stand as a crude reworking of an old piece like a middle school student submitting the same book report to two different teachers. In order to establish a finer link between CMS and Bacon, Eiseley added the following, brilliant, underlined segue: "How much more we would see, I sometimes think, if the world were lit solely by lightning flashes from the Elizabethan stage."[22] In reaching to Shakespeare (1564-1616), Eiseley seems to be drawing himself closer to Bacon, Shakespeare's long-rumored ghost author. Unfortunately, this connective tissue is not sustained. When Eiseley wrote at the outset of CMS, "What miraculous insights and perceptions might our senses be trained to receive amidst the alternate crash of thunder and the hurtling force that give a peculiar and momentary shine to an old tree on a wet night," he was referring to the stroboscopic cast the world would be given by real flashes of lightning. Towards the end of CMS, it is "a bolt of light that lit the man on the hayrick". The young man's mind flashes in response one menacing evening, but it is electric lightning throughout CMS, not theatrical lightning, that permeates the original essay. CMS does indeed dwell on the metaphorical lightning of a perfectly composed line of prose, or the metaphorical lightning of the inexplicable creative act, or the metaphorical lightning in the individual brain in which "there passes the momentary illumination in which a whole human countryside may be transmuted in an instant," but CMS works so well because these themes are bracketed at the start and the finish of the essay by real lightning bolts. It is actual lightning that grounds Eiseley's rhetorical flashes, "the light of the universe beyond our ken,"[2] and which distinguishes the rare individuals who can best see beyond their experience, from the rest of us. Bacon and Darwin were these preeminent seers in Eiseley's eyes.

In drawing his essay closer to Bacon through Shakespeare and the Elizabethan stage, Eiseley may also have been drawing himself closer to his father, an itinerant actor who recited Shakespeare to unsophisticated audiences in little Midwestern opera houses. Eiseley's father left his son a cherished, "thumbed copy of Shakespeare inscribed with his name."[19]

Bacon also was remembered warmly by Eiseley, even though this feeling was not universal in 1960. Lord Chancellor Bacon was convicted of accepting bribes late in his career, a scandal that led to the stripping of his official duties. During his last years, Bacon was a disgraced philosopher, writing alone. As Eiseley lectured on Bacon at institutions throughout the United States in 1961, he discovered a widespread animus directed at the memory of Bacon. Eiseley reflected in the volume that would last reproduce CMS, *The Man Who Saw Through Time*, that he found himself "embroiled…in sufficient controversy to make me wonder whether it was I who was threatened with the Tower and whether Parliament was in full cry upon my own derelictions."[30] Now, Eiseley, in his mind, *is* stepping squarely into Bacon's shoes.

The Slit

Time is preeminent theme in Eiseley's writing. At the outset of his autobiography, Eiseley is giving a lecture in a bright auditorium: "I started my speech. I was talking about time…All the sciences are linked by one element, time. It pervades them all."[19]

Time announced itself in "The Slit", the opening chapter of Eiseley's first book, *The Immense Journey*, that attracted of a broad audience.[4] Eiseley described a ride on horseback over flat ground until he reaches a sandstone outcrop. Here, he wrote, "I came upon the Slit," a crevasse worn by an ancient torrent. Eiseley worked his way into the Slit, "a perfect cross section through perhaps ten million years of time." There, he came face-to-face with the skull of a "shabby little" rodent – Hamlet like – inspiring a meditation on time:

> Perhaps the Slit, with its exposed bones and its far off vanishing sky, has come to stand symbolically in my mind for a dimension denied to man, the dimension of time…Out of it – forward or backward – he cannot run.

As Eiseley descended into the Slit, into the past, he looked up to see the sky becoming a narrower "slit [lowercase – not *the* Slit] of distant blue…already as far off as some future century I would never see." He impressed upon us that at any instant, we are forced to think about where we came from and where we are going. At any instant we can dig up the past, while being indifferently propelled into an uncertain future that with each passing moment of life becomes harder to achieve.

Wedged in the Slit, Eiseley could not go backward or forward corporeally but only by the use of his imagination. The best time travelers, Bacon and Darwin, were first among Eiseley's heroes because they could access the hidden psychological dimension. They were ultimately so dear to Eiseley, innovators he would return to so often, because they could transcend "the wound of time…the ability of the mind to extend itself across a duration greater than the capacity of mortal flesh to endure."[31] Darwin is the fossil collector, descending into the Slit, into the past, whereas Bacon is fixed on the crack of light belonging to the distant future. Darwin reckoned how we got here while Bacon foresaw the mechanics of modern science that would take humankind to unimaginable places. With the rat, Eiseley communed with Darwin and the flow of creatures "with little more consistency than clouds from age to age", while perseverating on the blue-sky future belonging to Bacon, a future that fossilized Eiseley would never see.

Any association of Francis Bacon with Charles Darwin had already been made by Darwin himself. On the flyleaf of Darwin's *On the Origin of Species*.[32] Darwin quoted Bacon from the *Advancement of Learning*:

> [L]et no man…be too well studied in the book of God's word, or in the book of Gods works; divinity or philosophy; but rather let men endeavour an endless progress or proficience in both.[33]

Darwin needed cover for work that he was certain would seem heretical to many. Bacon was a shield, but not only a shield. He was a model for challenging the prevailing epistemologies, ways of knowing. Bacon's less deft or less lucky contemporaries like Giordano Bruno (1548-1600) were burned at the stake for questioning those things that were created without room for dissent. Darwin and Bacon challenged the same God, one by looking backward and the other by looking forward, as did Eiseley, a self-described bone hunter who "spent a great deal of his life on his knees, though not in prayer."[34] Moreover, Darwin explained in his autobiography that his approach to natural philosophy was, in essence, an exercise in Baconian induction.

> [I]t appeared to me that by following the example of Lyell in Geology, and by collecting all facts which bore in any way on the variation of animals and plants under domestication and nature, some light might perhaps be thrown on the whole subject. My first note-book was opened in July 1837. I worked on true Baconian principles, and without any theory collected facts on a wholesale scale…[20]

Scholars have pointed out that Darwin was not purely Baconian.[35] Darwin tried, as he was collecting new facts, to fit all the pieces into preexisting ideas, some of which were discarded and replaced by others. Today, of course, we recognize that scientific invention, like essay writing, is messy.

The Ring

The solution to the mysterious substitution of Bacon for Darwin, a seemingly desperate act of an overcommitted author, is that for Eiseley, the time travelers Bacon and Darwin were twins merely facing in opposite directions along the dimension denied to ordinary philosophers. Eiseley's own flyleaf for *FBMD* captured the pairing of Bacon and Darwin, an acknowledgment, it seems to me, that what he wrote about Bacon was originally applied to Darwin.

> Sir Francis Bacon, the English philosopher and author, once spoke of those drawn into some powerful circle of thought as 'dancing (with)in little rings like persons bewitched.'[36] Our scientific models do simulate a sort of fairy ring, which, once it has encircled us, is hard to view objectively. In Charles Darwin's youth, the magic circle of fixity and that of organic novelty began to interpenetrate. The dancers bewitched by stable form discovered a new truth: evolution.[22]

FBMD is not about Darwin. Why should Charles be dancing on the front matter of this book?

Darwin appeared in a new way in the preface of the enlarged *FBMD*, the Bacon biography *The Man Who Saw Through Time*. Eiseley compared Bacon's efforts "to project for the masses a new definition of culture and inventiveness extending into the remote future" with the theory of natural selection, "as difficult a task as Darwin was later to encounter."[30] Of all the comparisons to Bacon's gaze into the future, Eiseley chose Darwin's gaze, two and one-half centuries later, into the past. Meanwhile, the "magic circle of fixity" passage, written for *FBMD*, appears on the opening page of Eiseley's posthumous book about *Darwin and the Mysterious Mr. X* (1979).[11] Why should Bacon also turn up as the leadoff batter in a book about Darwin, mirroring Darwin's cameos in the books about Bacon? None of it makes much sense until you realize that it didn't matter much to Eiseley, or his time travelers, for whom probity didn't matter much either.

Eiseley had already identified Bacon in *Darwin's Century* (1958),[37] written in advance of the centenary of *The Origin of Species*, as one of the first philosophers to articulate the idea of the survival of the fittest. He quotes Bacon and Darwin in sequence:

> Bacon: And it hath seldom or never been seen that the far Southern people have invaded the Northern, but contrariwise. Whereby it is manifest that the *Northern Tract* of the World, is in Nature the more Martial Region: Be it in respect of the Stars of that Hemisphere; Or of the great Continents that are upon the North, whereas the *South Part*, for aught that is known, is almost all Sea; or (which is most apparent) of the Cold of the Northern Parts, which is that which, without Aid of Discipline, doth make the Bodies hardest, and the Courages warmest.
> Darwin: I suspect that this preponderant migration from the north to the south is due to the greater extent of land in the north, and to the northern forms having existed in their own homes in greater numbers, and having consequently been advanced through natural selection and competition to a higher stage of perfection, or dominating power, than the southern forms.
> Eiseley: My intention in aligning thee two quotations is not, of course, to derive Darwin's biology from Bacon, but to give at least a glimpse of the antiquity of some of the ideas which needed only to be developed and elaborated in order to take a legitimate place in an evolutionary system of thought."[37]

Even more germane are Bacon's "evolutionary overtones" that Eiseley quoted in a passage in *FBMD*, not quoted in *Darwin's Century*.

> The transmutation of species is, in the vulgar philosophy, pronounced impossible, and certainly it is a thing of difficulty, and requireth deep search into nature; but seeing there appear some manifest instances of it, the opinion of impossibility is to be rejected, and the means thereof to be found out.[22]

Eiseley applauded Bacon for articulating such open-mindedness before properly extended geological time was appreciated.

He continues,

> [F]or the next two hundred years men allied in international societies originally foreseen by Bacon would make innumerable observations upon the strata of the earth, upon fossils, and upon animal and plant distributions. Heaps upon heaps of facts collected and combined by numerous workers would eventually lead to Darwin's great generalization."[22]

In part, Eiseley saw Bacon as having given Darwin necessary tools, and Darwin having reached back to acknowledge the debt – a virtuous, intellectually comprehensible ring. On the other hand, the ring, while it drew together favorite Eiseley themes, often came undone. Closing it was a lifelong aspiration. In setting up the switch of Darwin for Bacon in *FBMD*, Eiseley added a quote from Shakespeare absent in CMS:

> Within my soul there doth conduce a fight
> Of this strange nature, that a thing inseparate
> Divides more widely than the skie and earth.[38]

Eiseley leaned again on Shakespeare to do some more heavy lifting:

> Sir, in my heart there was a kinde of fighting,
> That would not let me sleepe.[39]

Like Hamlet, Eiseley's soul was also split and its components battled with one another:

> [T]here was only the ebb and flow of this formidable force, the creature which I could neither retreat from nor successfully confront. Through years it continued, a silent unseen duel…the conflict at the root of my being.[8]

One reviewer summed up Eiseley's autobiographical writing in this way:

> [It] is the record of the artist trapped inside the scientist. That sets up a unique struggle…the artist relating intensely personal, emotional, illuminating experiences that he perceives against the cold enormous edifice of scientific thought, which in turn is seen in relief against the overwhelming superstructure of time.[40]

Eiseley dedicated his first book about time, *The Immense Journey*, to his beloved father Clyde "who lies in the grass of the prairie but is not forgotten." [4] Eiseley's mother, Daisy, by contrast, is not remembered fondly. She was deaf and frequently howled, gesticulated urgently with her hands, or stamped her feet to communicate. Clyde, wrote Eiseley, "was a good man who bore the asperities of my afflicted mother with dignity and restraint."[1] Eiseley was fearful of Daisy's behavior, evidence of serious mental illness that haunted her side of the family and that might emerge in him someday. "My brain was so scarred," Eiseley recalled of his upbringing, "it is a wonder I survived in any fashion."[19]

It seems that Eiseley's solution to his "unseen duel", his professional self-expression, rooted perhaps in a divided household, was to defend the writer, Bacon, while killing off the scientist, Darwin – even though he admired Darwin immensely – so that he could let the writer thrive within. Carlisle hinted as much when he said that Eiseley's unique literary science "require[d] him in a way to renounce his scientific heritage."[41] This renunciation and the ensuing battle are displayed in Eiseley's work. Eiseley was the man on the hayrick, two halves uncomfortably welded together.

Inosculate

Eiseley, like Bacon, was known for his writing rather than his scientific discoveries. William Harvey (1578-1657), the discoverer of blood circulation, dismissively said that Bacon wrote like a Lord Chancellor. Harvey, according to Eiseley, thought Bacon was "a literary man who need not be taken seriously by historians of science".[1] It may have been that Bacon's masterpiece, *On the Dignity and Advancement of Learning,* "the first great prose work on a secular subject…in the English tongue,"[42] sounded too odd to Harvey's ears. Eiseley defended Bacon,

> That [he] was a writer of great powers no one who has read his work [except Harvey] would deny. He exercised, in fact, a profound stylistic influence both upon English writers who followed him and upon the scientists of the Royal Society. To say, for this reason, that he is of no scientific significance is to miss his importance as a statesman and philosopher of science...

Here, Eiseley is slipping between a defense of Bacon and of himself, also a writer of great powers "incapable of writing a dull or inelegant sentence."[43] In CMS, Eiseley recounted a visit by a serious young scientist who, "With utter and devastating confidence…had paid me a call in order to correct my deviations," following the publication of *The Immense Journey*,[4] "and to lead me back to the proper road of scholarship. He pointed out to me the time I had wasted – time which could have been more properly expended upon my own field of scientific investigations."[2] The young man stood for all those who gave Eiseley, the writer, a mixed reception. "Eiseley's literary accomplishments", according to his editor, "may have overshadowed his scientific endeavors and often confused those who did not understand what errand he was on."[43]

In time, Eiseley no longer wanted to continue writing technical papers. The spark of his transformation to a full-time essayist was an ear infection in 1956 that left him deaf for a prolonged period, albeit not permanently like his mother. The experience of having walked in his mother's shoes pushed him to cast off other burdens that he no longer wished to carry. During his silent year, Eiseley's decided that "from then on I would do and think as I chose."[44] Eiseley chose to write as he pleased, and he also chose to wage a battle against Charles Darwin.

As *Darwin's Century* went to press in 1958, Eiseley made some additional observations that could not be accommodated in the book. These observations were published in 1959 in a long, provocative essay in the *Proceedings of the American Academy of Arts and Letters*, "Charles Darwin, Edward Blyth, and the Theory of Nat-

ural Selection."[45] Eiseley charged that the theory of natural selection was foremost the insight of Edward Blyth (1810-1873), an amateur naturalist, and that Darwin knew of Blyth's insight but schemed to hide his knowledge of it.

Eiseley aligned various proofs that Darwin was dishonest in his presentation, "making unacknowledged use of Blyth's work."[45] First, Eiseley claimed that Darwin excised key pages from his first notebook on the species question that presumably acknowledged Blyth's idea. Second, Eiseley claimed that Darwin was intentionally misleading by suggesting that Thomas Malthus (1766-1834) was an inspiration for the idea that struggles in nature led to the persistence of advantageous variations. Rather than taking Darwin at his word, Eiseley insisted that Malthus was a deliberate foil behind whom Blyth was made to disappear. A third smoking gun is Darwin's telltale use of an unfamiliar word, "inosculate", that supposedly he had never used, until reading Blyth. Eiseley insisted that we, contemporary readers and students of science, by neglecting to acknowledge Blyth, and likewise Eiseley, are collectively "falsifying science history", preferring instead the easy narrative of an "inspirational flash" of a solitary genius.[45] This is a contradictory position for Eiseley who in CMS later prized the inspirational flashes of the solitary genius as the greatest lightning of all.

In October 1836, the *Beagle* returned to England after a four-year voyage of discovery, bracketed by two of Blyth's publications in *The Magazine of Natural History* of 1835[46] and 1837.[47] Eiseley showed with Darwin's own notebooks that the famed naturalist had received the 1835 *Magazine* when the *Beagle* docked in Peru. Darwin's own surviving 1837 *Magazine* contains annotations of Blyth's paper. Eiseley then reexamined Darwin's writing with Blyth in mind, something, he suggests "no one, it appears, thought of actually" doing.[45] Eiseley showed that Darwin frequently cited Blyth's prodigious knowledge of natural history, but suspiciously never the 1835 and 1837 papers. Eiseley believed that Darwin was hiding his knowledge of these papers.

In fact, the papers show that while Blyth discussed the transmutation of species, he was not an evolutionist, but rather a conservatist. Species adapted to environmental pressures, according to Blyth, so that "Providence [could] keep up the typical qualities of a species," a sentiment that Eiseley actually quoted.[45] "No notion of 'natural selection'" criticized Stephen Jay Gould, "could be more precisely contrary to Darwin's own."[48,49] Other critics of Eiseley's prosecution of Darwin had their say, emphasizing the failure of Blyth to see the creative potential of natural selection. There is a consensus that Eiseley got it wrong in broad strokes.[50,51,52]

There is no need to rehash this debate here. Much has been said already.[53] Rather, I emphasize that while Eiseley may or may not have been misguided with respect to the intentions of Blyth and Darwin, he was definitely wrong in the particulars. The smoking guns enumerated in the preceding paragraphs, it turns out, were cool.

1. *Notebook pages*. In 1960, the year of CMS, DeBeer and coworkers reassembled Darwin's notebooks after having relocated the missing pages.[54] There is no indication that Darwin conceded the paternity of natural selection in these pages to Blyth.[8] Darwin did not hide these pages, he cut them out and refiled them, as he was want to do in the course of his research.
2. *Malthus*. Subsequent research on the origins of *The Origin* showed that Darwin's alleged stimulation by Malthus, and others besides, was indeed earnest, not a smokescreen. Schweber[55] pinpointed the day, 28 September 1838, that Darwin read Malthus' *An Essay on the Principle of Population*.[56] When late in life[20] Darwin cited his inspiration in Malthus from October of that year, he was close enough. Perhaps it took a few days for the message to sink in.
3. *Inosculate*, according to Eiseley, is "[a] rare and odd word not hitherto current in Darwin's vocabulary suddenly appears coincidentally with its use in the papers of Edward Blyth…The rare and mildly archaic character of this word suggests that Darwin acquired it from his reading of Blyth." Blyth was fluent in the so-called quinarian taxonomy that was popular at the time, in which related organisms were grouped in rings intertwining or "inosculating," in the words of its inventor, to indicate the relationships of groups to one another. The quinarian system may have inspired Eiseley's Ring imagery. *Inosculate* is indeed rare and archaic to modern ears. Only a pedant would use it today in place of *intertwine*, a serviceable synonym, but at the time the *Beagle* returned to England, "inosculate" and "intertwine" were used with about equal frequency, after which insoculate began a steady decline. Google can now make such statements quantitative (Figure 1). Moreover, it was later shown that Darwin, in fact, had used inosculate in a letter of 1832.[51] *Inosculate* is not, as Eiseley contended, a word never in "wide circulation and which is not to be found in Darwin's vocabulary before this time."[11]

Nevertheless, Eiseley clung to the premise that Darwin stole from Blyth, even when Eiseley's key pieces of evidence no longer could be supported, and he reiterated his charge in several other places,[57,58,59] lastly in his auto-

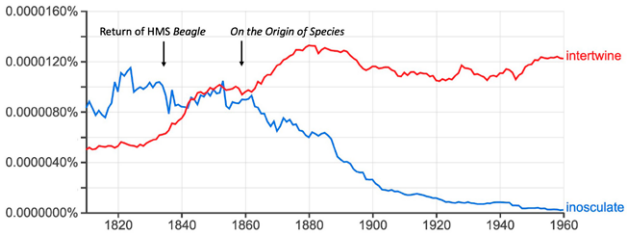

Figure 1. Google N-gram word frequency in % for "inosculate" and its synonym "intertwine" in British English from 1810-1960. Google smoothing function = 8.

biography: "Faintly the words of young Blyth whisper in our ears…"

> [I] hope that this endeavor will induce some naturalist, more competent than myself, to follow out this intricate and complicated subject into all its details…Be at peace, Edward. The man you sought came…I, who unearthed your whisper from the crumblings of the past, have been here and there excoriated by men who are willing to pursue evolutionary changes in solitary molar teeth but never the evolution of ideas.[19]

"Mere words," contended Eiseley in his dual defense of Bacon and self, which underlines his assault on Darwin, "can sometimes be more penetrating probes into the nature of the universe than any instrument wielded in the laboratory."[22,30]

In 1968, Eiseley was interviewed about the charges he leveled against Darwin, and he was asked in particular why Blyth stands in Eiseley's estimation as so far above the many other forerunners to the idea of evolution from whom Darwin might have taken inspiration,[60] including Darwin's own grandfather, Erasmus. The interviewer reported that "Eiseley was silent…[and gave the impression that he] did not keep abreast of new Darwin research".

> As the evidence steadily accumulated that Darwin's discovery of natural selection was complex and was determined by many authors – and that Blyth's influence was unexceptional – Eiseley simply kept on stating that it was Blyth who held the 'vital keys'…[61]

Eiseley was demonstrably wrong about three pillars of his argument, but he would not allow for the possibility that he was misled in other ways. He was inflexible. His protracted battle against Darwin – and he loved Darwin – was entangled with questions of his own identity, and human beings will go to extremes to preserve – and maybe sometimes destroy – our identities.

CONCLUSION. EISELEY AND RICHARD NIXON

By 1969, Eiseley was no longer provost. That year Penn, like many American universities, was rocked by anti-Vietnam War protests. The activism was unsettling to Eiseley. His *alma mater* had become unfamiliar. Eiseley remarked that his thesis advisor would have "died of frustration if he had had to face the students of the sixties."[19] In a letter to *Science* called "Activism and the Rejection of History", Eiseley characterized "an extremist minority" on campus deliberately abandoning history at the expense of an "absurd, degrading, and irrelevant" moment. Eiseley composed another letter, unearthed by Christianson,[8] "pleading" to President Richard Nixon: "[M]any people, particularly the young, are the more or less innocent dupes of unseen elements making use of the mass media for the purposes of propaganda" and he urged Nixon to "retard the uncomfortable ebbing away of our power and purpose."[62] Eiseley was on "the wrong side of the generational tracks" according to one historian.[63] The next year, as accolades continued to accumulate for Eiseley, he was invited to be the commencement speaker at Kent State University in Ohio. The ceremony was canceled after the National Guard, on 4 May 1970, fired upon student protesters, killing four and wounding ten others. Eiseley was quoted in the *Philadelphia Inquirer* of having said to a friend of the protesters: "They got what they asked for."[64]

I was shocked to read this reported comment from a writer known for his grace. This was the dark side of his (our) twofold countenance. The dismissal of slaughtered students, among those he had been scheduled to send into the world with bright words, was a sentiment that is at odds with the empathy that Eiseley could direct at the skull of a long dead Paleocene rat.[4]

Eiseley's opinions over his career are riddled with contradiction. When a Japanese-American friend faced discrimination in the run up to World War II, Eiseley told his companion, "If one man can apologize for a nation, his nation, I apologize." In the next breath, he laments a "menacing and mocking" new third world in the United Nations. His friendship is contrasted to later experiences with "embittered and truculent minorities."[19] The conflicting sentiments in just one book are sometimes head spinning.

That said, Eiseley was aware that he was a citizen of his time: "A man [or woman] comes into life with certain attitudes and is inculcated with others of his time. Then some fine day, the kaleidoscope through which we peer at life shifts suddenly and everything is reordered."[19] Eiseley, the author who wrote of Bacon and Darwin sailing through the ages in their minds, couldn't even put himself one generation into the future.

Naturally, there are things that are always easier to recognize in others than in ourselves. That's obvious, but urgent enough in the present moment of social change that it is not redundant for the illustration. "[I] do not pretend to have set down, in Baconian terms," Eiseley wrote,

> a true and consistent model of the universe. I can only say that here is a bit of my personal universe, the universe traversed in a long and uncompleted journey. If my record, like those of sixteenth century voyagers, is confused by strange beasts or monstrous thoughts or sights of abortive men [the "double face of man," "diseased and fungoid"[4]], these are no more than my eyes saw and my mind conceived.

ACKNOWLEDGMENTS

I am grateful to the Yale University Archives, Beinecke Rare Book and Manuscript Library for access to the Rachel Carson archives. Financial support was provided by the National Science Foundation, DMR-203968.

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Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Historical Article

New Insight into the "*Fortuitous Error*" that Led to the 2000 Nobel Prize in Chemistry

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Abstract. In 2000, the Nobel Prize in Chemistry was awarded to Hideki Shirakawa, Alan G. MacDiarmid, and Alan J. Heeger "for the discovery and development of electrically conductive polymers." While this award was in reference to their collaborative efforts on conducting polyacetylene in the mid-to-late 1970s, the narrative leading up to these efforts began in 1967 with the production of polyacetylene plastic films via what has been called a "*fortuitous error*." At the heart of this discovery were Shirakawa and a visiting Korean scientist, Hyung Chick Pyun. The current report provides background on Pyun and, for the first time, presents his version of the events leading to the discovery of polyacetylene films in order to provide new insight into this important historical event.

Keywords: polyacetylene, plastic films, Nobel Prize 2000, polymerization conditions, Ziegler-Natta catalysis.

OPEN ACCESS

Citation: Rasmussen S. C. (2021) New Insight into the "*Fortuitous Error*" that Led to the 2000 Nobel Prize in Chemistry. *Substantia* 5(1): 91-97. doi: 10.13128/Substantia-973

Received: Jun 22, 2020

Revised: Aug 18, 2020

Just Accepted Online: Aug 23, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

INTRODUCTION

In 2000, the Nobel Prize in Chemistry was awarded to Hideki Shirakawa, Alan G. MacDiarmid, and Alan J. Heeger (Figure 1) "*for the discovery and development of electrically conductive polymers*," which was in reference to collaborative efforts by these investigators on conducting polyacetylene in the mid-to-late 1970s.[1-9] The narrative leading up to these collaborative efforts, however, began in 1967 with what Shirakawa has referred to as a "fortuitous error," an event that resulted in the very first production of polyacetylene in the form of plastic films.[10-13] According to Meriam-Webster, a legend is defined as "a story coming down from the past; especially: one popularly regarded as historical although not verifiable". The story behind the critical discovery that polyacetylene could be synthesized as lustrous, silvery films has achieved such legendary status, with it having been told and retold by many different people over the years, with the story rarely told the same way twice.[10-29]

What is generally agreed upon is that this discovery was made in October of 1967,[10-15] approximately a year and a half after Shirakawa had joined the group of Sakuji Ikeda (1920-1984) at the Tokyo Institute of Technology (Tokyo Tech).[14] According to Shirakawa,[10-15] another researcher under his supervision was preparing a sample of polyacetylene, but mistakenly used a

thousand-fold excess of catalyst. The result of this error then caused the formation of "*ragged pieces of a film*"[14] on the surface of the catalyst solution, rather than the typical black powder normally produced within the solution.[30-41] Although interest in the electronic properties of polyacetylene date back to the 1958 report of Giulio Natta (1903-1979),[32] studies prior to the discovery of polyacetylene films were limited to power samples, typically as pressed pellets.

At the same time, however, there are a number of variable aspects given in the many retellings of this event, including the identity and nature of the researcher who made the critical error. While the researcher's gender is generally viewed as male, he has been described by various sources as a "student",[16,19-21] "Shirakawa's student",[16] "foreign student",[19] "graduate student",[16,18,21] "Korean graduate student",[21] "visiting Korean researcher",[20] "Korean visitor",[22] or "visiting scientist".[14] Another variable point is the specific reason for the error itself. While most agree that it was the result of miscommunication, the nature of the miscommunication differs even within the accounts of the three Nobel laurates. For example, Shirakawa states[14] "*I might have missed the "m" for "mmol" in my experimental instructions, or the visitor might have misread it*," while MacDiarmid gives a different account,[19] stating "*I asked him how he [Shirakawa] had made this silvery film of polyacetylene and he replied that this occurred because of a misunderstanding between the Japanese language and that of a foreign student*". Although somewhat similar to that of MacDiarmid, Heeger gives still yet another version,[22] stating "*he [Shirakawa] had a Korean visitor who misunderstood what he said in Japanese.*"

It is only in the acknowledgment of his Nobel lecture that Shirakawa finally reveals the name of the researcher at the center of this event to be Dr. Hyung Chick Pyun (Figure 2).[10-13,15] Pyun was never included as a co-author on any of the papers on the synthesis of the polyacetylene films, although the initial 1971 report included an acknowledgment to "H. C. Pyun".[42] Other than that, very little is known about Pyun and it is only recently that some biographical data has been reported.[28] The goal here is to provide background on Pyun and, for the first time, present his version of the events leading to the discovery of polyacetylene films in order to provide new insight into this event.

HYUNG CHICK PYUN

Hyung Chick Pyun (Byun Hyung Jik; Byeonhyeongjik) was born December 23, 1926 in Bongsan county of Hwanghae province, now within North Korea.[43] His family moved to Seoul in 1936,[44] where he was educated at Kyungdong High School.[43] In April of 1945, he entered the Sixth High school in Japan (which become part of Okayama University in 1949), before returning to Seoul in October 1945 to enter Kyungsung University's preparatory school.[44] Kyungsung University became part of Seoul National University in 1946, were Pyun completed a B.S. in Chemical Engineering in 1951.[43] With the onset of the Korean War (1950-1953), he began working for the Science Research Institute of the Ministry of National Defense in December of 1950, while also completing his university studies.[43,44] He continued there

Figure 1. Hideki Shirakawa (b. 1936), Alan G. MacDiarmid (1927-2007), and Alan J. Heeger (b. 1936) [Reproduced from Ref. 20 with permission of the Royal Society of Chemistry].

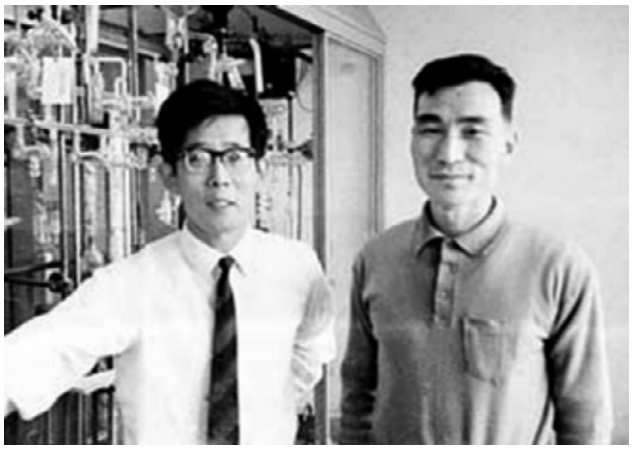

Figure 2. Hideki Shirakawa and Hyung Chick Pyun (1926-2018) at Tokyo Tech in 1967 [Courtesy of Joongmoo Byun].

until 1960, when he moved to the newly established Korea Atomic Energy Research Institute (KAERI).[43] Beginning in February of 1961, he spent a year at the University of Kansas,[43,44] where he worked with William E. McEwen (1922-2002).[28] He then published his first papers in 1964,[45,46] the first of which was based on his work with McEwen.[45] In 1967, he received support from the International Atomic Energy Agency (IAEA) to carry out research in Japan.[43,47] Thus, from May 1967 to the following March, he worked in Tokyo on a joint project between Sakuji Ikeda (1920-1984)[28] at Tokyo Tech and Yoneho Tabata (b. 1928) of the Nuclear Engineering Department at the University of Tokyo.[44,47,48] Although it has been reported that Pyun had acquired his doctorate before working in Ikeda's laboratory,[24] this is incorrect and he received his Ph.D. in Nuclear Engineering from Seoul National University in 1970, based on work he had published in 1964.[43,49] Although he expressed a desire to pursue polyacetylene research after his return from Japan, he was discouraged to do so by his superiors. As such, the only publication that appears related to his time in Tokyo was a 1969 report on the comparison of gamma irradiation vs. Ziegler-Natta catalyzed methods for the copolymerization of phenylacetylene and styrene.[50] However, his work turned primarily to polymeric materials and composites after that point, which seemed to be the focus of this research for the rest of his career. Pyun retired from KAERI in 1991[43] and died on March 8, 2018 after an extended illness.[44]

PYUN'S ACCOUNT

While previous efforts to obtain Pyun's version of the events had been unsuccessful,[24] the collaborative approach utilized here included a native researcher with access to Korean-language resources less available to western historians. Thus, it was confirmed that Pyun was still alive as of late 2017, although quite ill and had been in the hospital since the fall of 2016. While we were unable to talk to Pyun directly, his son Dr. Joongmoo Byun was quite helpful and provided us with a written account his father had prepared prior to his failing health. This document, roughly entitled "What is a Nobel Prize?" in English, was last revised in 2013 and detailed his memories and views on the discovery of polyacetylene films (see Supporting Information for the original Korean document and a working English translation).[47] A previous version of this account had also appeared in a KAERI publication in 2002.[48]

According to Pyun, he arrived at Tokyo Tech in 1967 to begin work on a collaborative project between Sakuji Ikeda and Yoneho Tabata (University of Tokyo), with the goal of studying the copolymerization of ethylene and tetrafluoroethylene (TFE) via the IR analysis of isotopically labeled species. The needed deuterated ethylene was to be prepared in Ikeda's laboratory, which would then be copolymerized with TFE at the University of Tokyo. Pyun had successfully completed the work in Ikeda's lab, but his work at the University of Tokyo was postponed as Tabata was visiting the United States and his return was delayed. During the month wait, Pyun became interested in the polyacetylene studies carried out by others in Ikeda's lab and felt that its properties could be improved if larger polymer particles were generated.[47,48] He proposed that this could be accomplished by decreasing the stir speed during polymerization and began investigating this. Pyun stated that:[47]

> One day…the stirring motor stopped during the experiment because the stirring speed had been excessively reduced. I was very embarrassed at first, but after a closer look, I found it surprisingly to see a silver film on the surface of the reaction solution. It was nothing more than a polyacetylene film... I realized that the scientists who were studying this field had not synthesized the acetylene in the film state because the polymerization reaction had proceeded with stirring. That is, if it is not stirred, it is allowed to polymerize in the film state. Stirring thereby was hindering film formation.

Pyun stated that he repeated the film production more than 10 times and thought that Ikeda would be very pleased, so he went to his office and gave a verbal report of his results. However, according to Pyun,[47,48] Ikeda became upset and reminded him of the joint project with Prof. Tabata, who had now returned from the US. The following day, Pyun was then sent to Tokyo

University to finish his project there. Weeks later, Pyun returned to Ikeda's lab in order to collect a deuterated ethylene sample, at which point Shirakawa asked him to demonstrate how to make the film. According to Pyun,[47,48] he showed Shirakawa his method in detail, after which Shirakawa was able to reproduce his results. Meanwhile, the research in Tabata's lab was proceeding smoothly, but due to the initial delay, Pyun was short on time and decided to apply for an extension to the IAEA. This required a recommendation letter from Ikeda, however, which he refused to give, thus denying any extension.[47] Pyun then returned to Korea in March of 1968.

DISCUSSION

In his written account,[47] Pyun highlighted a number of issues he had with the accepted version of the accidental discovery. This included statements that described him as only a graduate student, that said he that did not know Japanese well, and that said he did not follow directions, specifically that he had used a thousand times too much catalyst. The first two of these were indeed incorrect, as has been previously pointed out by select historical studies of these events.[24,26,28] As given above, although he did not yet have his Ph.D. during his time in Ikeda's lab, the research that became his dissertation had already been completed and Pyun was already 17 years into his professional career at the time. In a similar vein, the issue of language had already been disproven by Hargittai,[24] who had confirmed with Shirakawa that Pyun had grown up in Korea during the years that the country was under Japanese occupation and thus spoke fluent Japanese. To be fair, however, Shirakawa had never described him as a student, nor had he ever said that Pyun's Japanese was a limitation and both of these points had been introduced by others during the many retellings of these events.

The final point, however, is more problematic. According to Pyun, he did not make any errors in the experimental conditions, did not use excess catalyst, and had purposely reduced the stirring rate, which resulted in the formation of the polyacetylene films.[47,48] As such, he felt that he had been unfairly denied the credit for the discovery, which in his view was not an accident. The view that this innovation was solely the result of reduced stir rate, however, is not consistent with the wealth of evidence to the contrary. The production of linear, conjugated polyacetylene dates back to the 1955 work of Giulio Natta (1903-1979), who had used very similar conditions to that of Ikeda's group.[30-32] More critically, Natta's original polymerizations were performed both with and without stirring, but always giving a crystalline powder, not a film. Furthermore, the critical requirement of catalyst concentration for film formation was independently confirmed by multiple groups after Shirakawa and Ikeda finally reported the detailed experimental procedure in 1974.[28,51] Finally, in 1987, Herbert Naarmann at BASF, along with coworkers from the University of Montpellier, published a paper that probed in great detail the effect of polymerization conditions on the properties of the resulting polyacetylene.[52] This study too was done without stirring and concluded that the film density was directly related to the catalyst concentration, with true films only formed via the application of high catalyst concentrations. As such, there is no experimental or literature support for Pyun's belief that he did not use a thousand-fold excess of catalyst in the original experiments. At the same time, it should be pointed out that his insistence that the reaction not be stirred does play a role in this process. As the film is formed at the gas-solvent interface, an unstirred solution provides a calm, undisturbed surface optimal for the production of smooth, uniform films, and it is perhaps not coincidence that Pyun did not observe film formation until the stirrer failed.

While there has never been any doubt that Pyun was the first one to prepare polyacetylene films, the addition of his version to the previously available accounts now allows some additional insight into these events. From Pyun's own accounts, he began performing acetylene polymerizations, but seemingly with limited knowledge of the existing polyacetylene literature at the time,[28] or even the wealth of such work carried out at Tokyo Tech.[33-41] Furthermore, based on Ikeda's reaction when Pyun reported his results and Pyun's own insistence that Shirakawa did not supervise his polymerizations, it appears that he did not have permission to carry out these experiments. After Pyun had been expelled from the lab, Shirakawa was then tasked with figuring out what Pyun had done, but could not reproduce his results. While it is not currently possible to confirm this, it appears that Pyun's notebook did not indicate the atypical catalyst concentration and it was only after witnessing Pyun perform the experiment that the unusual amount of catalyst came to light, after which Shirakawa was then able to reproduce the results. Shirakawa was then the one that studied the process in detail, after which he able to provide an accurate account of how the polyacetylene films were being produced, as well as detailed studies of the polymer structure, film morphology, and resulting electronic and optical properties. While it is very unfortunate that Pyun felt he was denied fair credit, this new insight does allow us to finally

understand Pyun's role in these events. His actions did result in the first formation of polyacetylene films, but the lack of understanding of what he did makes it difficult to give him sole credit for discovery as he desired. Still, many researchers would have probably made him a coauthor on the first paper reporting the film formation, rather than just an acknowledgement.[42] Of course, the specific criteria for determining authorship can vary by both discipline and research group, and deciding what merits authorship is not always a straightforward process.[53-55] Furthermore, it is important to remember that while Shirakawa is always the focus when discussing this work, he was only a research associate at the time and would not have been the one to determine authorship. Rather, it was Ikeda who was the principle investigator and the decision concerning authorship would have ultimately been his to make.

Finally, some may feel that Shirakawa's version of the discovery was an attempt to distort the facts or cover up what happened. Here it is essential to separate the legend from verifiable statements, recognizing that while many have attributed comments to him, the truth is Shirakawa has actually said very little on the subject and what has been said is somewhat vague. Unfortunately, this has led others to fill in the details based on their own preconceptions, thus leading to the multiple and erroneous versions of this important event.

ASSOCIATED CONTENT

Supporting Information: Pyun's original account in Korean, as well as a working English translation.

ACKNOWLEDGMENT

I would like to thank Joongmoo Byun for providing significant material on his father, as well as Choon H. Do, whose collaboration made this project possible.

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Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Historical Articles

Udagawa Youan (1798-1846), Pioneer of Chemistry Studies in Japan from Western Sources and his Successors

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OPEN ACCESS

Citation: Siderer Y. (2021) Udagawa Youan (1798-1846), Pioneer of Chemistry Studies in Japan from Western Sources and his Successors. *Substantia* 5(1) : 99-117. doi: 10.36253/Substantia-963

Received: Jun 08, 2020

Revised: Sep 06, 2020

Just Accepted Online: Sep 14, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Abstract. This work presents chemistry studies of the Japanese scholar Udagawa Youan (1798-1846), specifically, his pioneering book *Seimi Kaiso*, introduction to Chemistry, and includes a short biography of Youan. The first aim of this work is to present Youan's contribution to Western chemistry in Japan. Youan studied many Western books and listed their authors. The new terms he invented for chemistry in Japanese influenced the development of chemistry writing and application in Japan. The seven books of *Seimi Kaiso* that were published during 1837-1847 and republished with annotation in Japanese in 1975 are discussed in this article. The impact of Youan' terminology on the history of chemistry writing in the nineteenth and twentieth centuries is discussed. The conditions of knowledge transfer among Japanese and Western scholars were very different. Youan had severe difficulties facing the strict attitude of the Tokugawa authorities toward studying and distributing knowledge coming from foreign countries. The later development of Japanese chemistry language and studies is also described.

Keywords: Japan, Udagawa Youan, *Seimi Kaiso* – Introduction to Chemistry, Western sources of science, Dutch Studies in Japan, Japanese chemistry terminology.

1. UDAGAWA YOUAN (1798-1846) – SCHOLAR OF DUTCH STUDIES

Udagawa Youan– A multi-talented nineteenth century scholar

Udagawa Youan (1798-1846) was a scholar of many talents who touched very many topics during his lifetime.[1] Youan was a medical doctor of Tsuyama town in Okayama prefecture, translated and investigated plants in Edo Japan, studied modern chemistry and many other topics like musical instruments, geography, history of Holland and playing cards; he wrote an early article on coffee and more. Youan studied foreign languages, first Dutch, to some level German, even Latin and Greek Russian, and copied a list of Arabic letters. It is told that in 1822 he stayed on a British ship for three nights in order to learn English. He saw maps of the world from which he could study names of European and other countries.[2]

Figure 1. Scholar Udagawa Youan.

In his youth, Youan studied Chinese Classics in the house of his teacher and adoptive father Udagawa Genshin. An official translation office of the Tokugawa regime, *Bansho wage goyo* was established in 1811; Youan joined the translation office in 1826. Youan collaborated there in Dutch translation with his adoptive father, his teacher Baba Sajuro, Otsuki Gentaku who was one of the founders of Dutch Studies and other Japanese scholars. They translated parts of Chomel encyclopedia, from which Youan learnt about Western botany.[3] Youan's good knowledge of Chinese Classics and good knowledge of Dutch language that he acquired due to years of translating books on plants, botany, medical drugs, and other topics, helped him understanding and coining suitable terms in Japanese for the new discipline, chemistry: names of the chemical elements, compounds, and chemical processes. Youan's innovation of scientific language remains in use today.

2. *SEIMI KAISO* – INTRODUCTION TO CHEMISTRY – YOUAN'S BOOK AND ITS CURRENT RESEARCH

Youan's main book on chemistry *Seimi Kaiso* will be dealt with in the following chapters. Before this book he wrote several other, shorter books on various chemical topics translated from the scientific books imported to Japan. A thorough survey in archives was carried out by J. Mac Lean searching the years 1712 – 1854. He studied the records of the Dutch Factory in Japan, and from the Colonial records, both preserved in the *Rijksarchief* (State Archive) in The Hague, the Netherlands. Mac Lean listed the year that a ship arrived, its name, its captain's name, the scientific instruments and books that were imported; the names of those who ordered those items are also listed.[4] Udagawa Youan might have had access to some of those books and instruments, especially those delivered to the official translation office whose member he was since 1826.

A partial list of U. Youan's early chemistry books includes: Metal Chemistry, Introduction to Chemistry Characters Sound, Dyeing Chemistry, Earth Chemistry number 1, Chemistry of Light, Element Earth (non-metal) Chemistry, Consideration of Western Measures, Note on Western Mineral Springs, Introduction to Chemistry Sequel Potassium Nitrate Theory, Theory on Hot Springs Experiments in Several Provinces.[5]

Description of Seimi Kaiso

Udagawa Youan's *Seimi Kaiso* is considered the first extensive book on chemistry in Japan. It includes seven books; each divided into three volumes and numbered chapters. Six books are considered inner books that are the main text; the seventh book is called an external or appendix book. All together it has more than 1100 pages, published between 1837 to 1847. The print is in Kanji and katakana. The books are bound by ribbon with several stiches along the back of the book. The pages are folded and numbered on their margin. Fig. 2 presents a full set of seven books in an original book case at the Edelstein Collection of The National Library of Israel (NLI) in Jerusalem. Supposedly, it was bought by Dr. Sidney Edelstein from a books shop in New York.

The first page of the first book is presented in figure 3. The upper line, written from right to left, shows the year of printing, corresponding to 1837. On the upper right side is written "Udagawa Youan translator." *Seimi Kaiso* 舎密開宗 are the four large letters in the middle of the figure. *Seimi* 舎密 meaning "Chemistry" follows the sound of the Dutch word *Chemie*. The word for chemistry was changed to *kagaku* 化学 meaning "the study of change" after the Chinese term.[6] There is a written warning against forgery on the left lower first page of each book.[7]

In *Seimi Kaiso* Youan dealt with topics such as chemical affinity, solution, caloric, alkali, salts, phosphoric acid, ammonia, oxidation and reductions of metals, glass, constituents of plants and more. Youan studied the ingredients of water in hot springs in Japan and

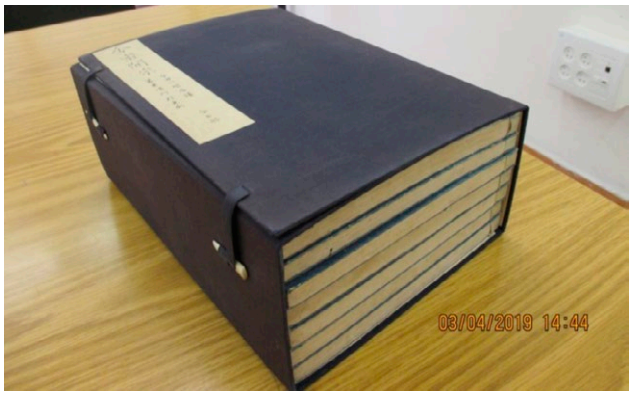

Figure 2. *Seimi Kaiso* set of 7 books at Sidney Edelstein Collection of the History of Science, The National Library of Israel (NLI) in Jerusalem. Photo: Y. Siderer.

Figure 3. *Seimi Kaiso* first page of the first book. Photo: Y. Schley.

described chemical ingredients of hot springs in foreign countries. Udagawa Youan cited fifty-eight elements, five of them were found to be mistakes, among those are caloric and light.[8] The chemistry studies that Youan started continued after him, some of the chemistry terms that he coined are still in use, see below, chapter 4.

In 1975 Youan's *Seimi Kaiso* was rewritten in modern Japanese, including translators' comments. *Seimi Kaiso* is based on about 24 chemistry books from Europe of late eighteenth and early nineteenth centuries. The revised text is written in Kanji, hiragana, and katakana, the last one is used for foreign names of places, people and chemicals. This volume, *Seimi Kaiso Research* holds 570 pages, in a hard black cloth cover, a paper cover and a book case. The book is opened from right to left, as are the original *Seimi Kaiso* books. Editor and preface writer is Tanaka Minoru. Each page shows the original book on its upper part and its currently rewritten text below it, (figures 4 and 5). It contains index of foreign names, index of Japanese materials, photos of several of Youan's apparatus drawings and copies of relevant books' covers. It also contains conversion tables of units of length and volume (p. 542) and weight (p. 543).[9] The main *Seimi Kaiso Research* book (hence SKR) is followed by a second book, written by Tanaka Minoru, Sakaguchi Masao, Dōke Tatsumasa and Kikuchi Toshihiko, with articles on Udagawa Youan, his life, work and his diary. This book of articles will be referred to as *Seimi Kaiso Articles* (hence SKA).[10]

Western Books that Youan Studied

Japanese and Dutch scholars tried to find out the original books from which Youan received his knowledge. In the introduction to *Seimi Kaiso* Youan wrote the names of the authors of the books he studied from and his translation of the title of the book, in Japanese. In Figure 4 a circle ◯ marks the beginning of a book or author's name.

Japanese scholars searched the books left by Youan and tried to match his Japanese writing with the Dutch books found in his house, or in the house of other scholars of Dutch studies. Tsukahara Togo observed that "Youan must have been able to use those manuscripts because he occupied one of the most privileged position in the *Rangaku* society as the member of the Udagawa family and also through his official function in the translation bureau, he was supposed to have wide access to the Dutch sources. In Holland, the identification of the original works of *Seimi Kaiso* was attempted in 1858 by J. J. Hoffmann (1805-1878), the first professor of Japanese studies in Leiden that started in 1855, and later by Serrurier (1846-1901), curator of museum of ethnology in Leiden." Their work relied on deciphering the phonetical transcription of the author's name and the modified Western book title.[12]

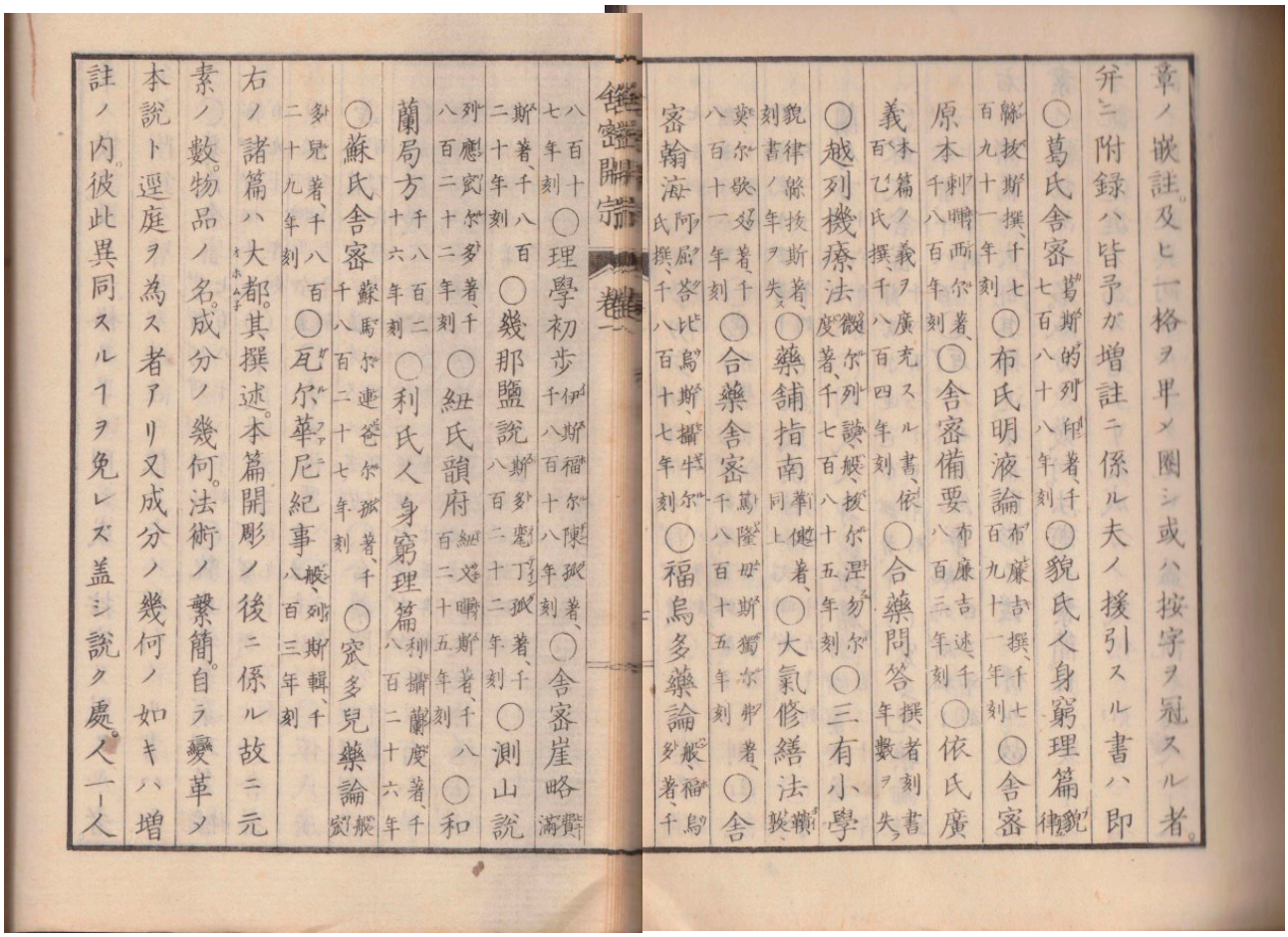

Figure 4. Names of Western authors and books in Udagawa Youan's *Seimi Kaiso* book 1 vol. 1 p. 7.[11] Photo: Y. Schley.

The List of Western Books and their Authors

Following is the list of authors as they appear in the original first book of *Seimi Kaiso* (Fig. 4), hence shortened SK.[13] The list presents the following authors names. Kasteleyn, P. J. (1746-1794), Blumenbach, J. F. (1752-1840), Plenck, J. J. (1735–1807), Lavoisier, A. L. (1743-1794), Ypey, Adolph (1749-1822), Niewenhuis, G., Bernvald, William van (1747-1826), Hagen, K. G. (1749–1829), Guiton de Morveau L. B. (1737-1816), Trommsdorff, J.B. (1770-1837), Ségur O. (1779-1818) , Houte, H. J. (1789-1821), Isfording, J. J. (1776-1841), Hijmans, H. S., Stratingh, E. (1804-1876), Reinwardt, C. G. C. (1773-1854), Dutch Pharmacopeaia 1826, Richerand, A. (1779-1840), Catz Smallenburg, F. van, Water, J. A. van de (1800-1832(?)), Rees, W. Van (1752-?).

Detailed descriptions of authors' names, their book or books and Western book source are presented in appendix 1, including: Author's name in English. Book's title in English, Japanese book's name in kanji, Japanese name in English letters, Japanese book title in English. Dutch book title. Book title in its original language in case there is one; further details and explanations. Youan wrote a shortened name for the authors he cited, in which the first syllable of the author's name is written before the book's title.

In some of the citations Youan mentions studying the book he had, in order to study another chemist whose book he did not have. These include citation of his European contemporary scientists e.g., Berzelius (1779-1848), Davy (1778-1829), Dulong (1785-1838), Gay-Lussac (1778-1850) and others. So actually he studied more than the books listed above and from those he chose which text and authors to cite.

Scholars cited by Youan from books not present in *Seimi Kaiso* list, include (not inclusive, there are more than 160 names of authors): Wedgewood Josiah, Empedocles, Cavendish Henry, Gaubius Hieronymus D., Gadolin Johann, Kirwan Richard, Gmelin Leopold, Glauber Johann R., Klaproth Martin H., Gay-Lussac Joseph L.,

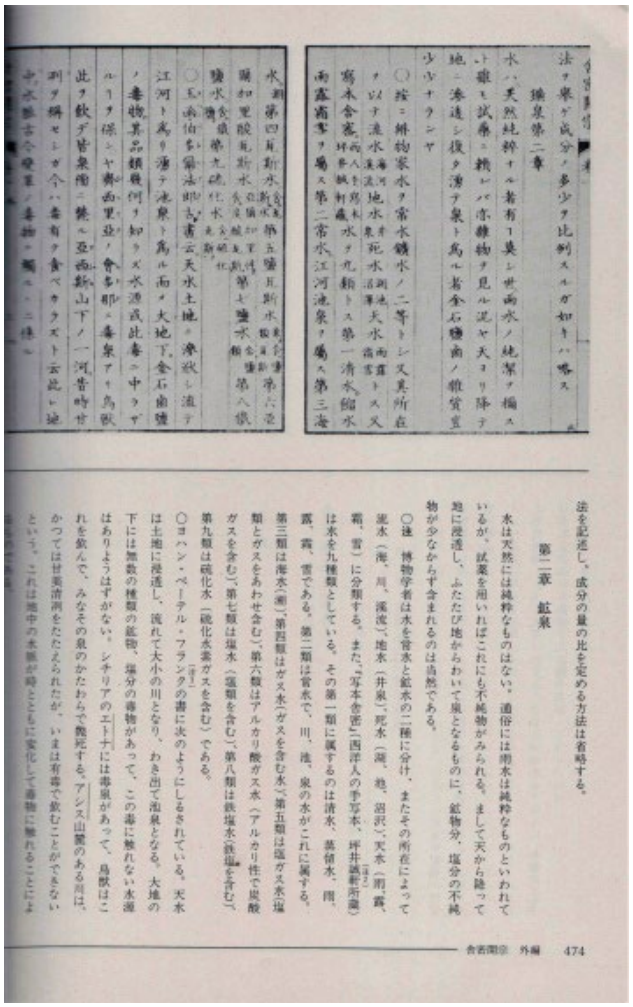

Figure 5. Upper part: *Seimi Kaiso* external book vol. 1, ch. 2 on mineral spring. Lower part: *Seimi Kaiso Research* p. 474. The first lines of the text on water impurities are dealt with in chapter 3 below. Photo: Y. Schley.

Scheele Carl W., Chaptal Jean A.C., Stahl George E., Seignette Pierre, Saussure Horace B. de, Thales, Davy Humphry, Döbereiner Johann W., Dulong Pierre L., Thomson Thomas, Hatchett Charles, Buffon Georges L.L., Faraday Michael, Black Joseph, Priestley Joseph, Fourcroy Antoine F. de, Proust Joseph L., Bergman Torbern O., Berzelius Jöns J., Berthollet Claude L., Boyle Robert, Hoffmann Friedrich, Beaumé Antoine, Homberg Wilhelm, Ure Alexander, Richter Jeremias B., Linné Carl, Rinman Sven, Lemery Nicolas, Rosello Hieronymus.[14]

We see the wide investment of Youan's chemistry study from books from the West, from original books in Dutch, and from books translated into Dutch from French, German, Latin, and Swedish. Most of the books were written in the late eighteenth or early nineteenth century, so Youan studied books that were about forty to ten years old in time of many new discoveries in chemistry. This could have led to his difficulty in understanding texts that were not clear or erroneous, or better understood in the West later.

The chemistry studies that Youan started continued after him, some of the chemistry terms that he coined are still in use, see below, chapter 4.

In *Seimi Kaiso* Youan dealt with topics such as Chemical affinity, solution, saturation, heat element, caloric. Gas, oxygen, nitrogen, atmosphere, hydrogen, water. Alkali, ammonia, acid, carbon. Youan addressed salts, sulfur and nitric acid. In the third book he addressed acids like phosphoric acid, boric acid, fluoric acid, and some metal compounds like barite, strontia and zirconia. Metals like gold, silver, iron, mercury, copper, lead, tin, zinc, bismuth, antimony, mangan, cobalt, nickel, and others were discussed. Organic acids like oxalic acid, citric acid, gallic acid, apple acid, tartaric acid, benzoic acid were studied. Youan wrote about soap, oils, resin, camphor, fiber, pigments and wax. In the last, external book, he wrote about analysis of mineral water, vegetable pigments, classification of springs and artificial preparation of mineral water.[15]

Tanaka Minoru devoted articles to Youan's perception of chemistry in *Seimi Kaiso* and discussed Youan's misunderstanding and mistranslation.[16] The question why Youan did not include the discussion about "atom" deserves further study.

3. EXAMPLES OF YOUAN'S TRANSLATIONS FROM WESTERN SOURCES

Four examples of Youan's studies are presented: 1. Henry-Youan: Water chemical ingredients analysis. 2. Galvani column. 3. Nitrogen oxides compounds. 4. Hot springs abroad and in Japan.

William Henry (1774-1836) text on Water Analysis and Udagawa Youan's translation

Examination of Mineral Water by Re-agents

Henry: Water is never presented by nature in a state of complete purity. Even when collected as it descends in a form of rain, chemical tests detect in it a minute proportion of foreign ingredients. And when it had been absorbed by the earth, had traversed its different strata, and is returned to us by springs, it is found to have acquired various impregnations. The readiest method of judging the contents of natural waters is by applying what are termed tests or re-agents, i.e. substances which on being added to a water, exhibit, by the phenomena they

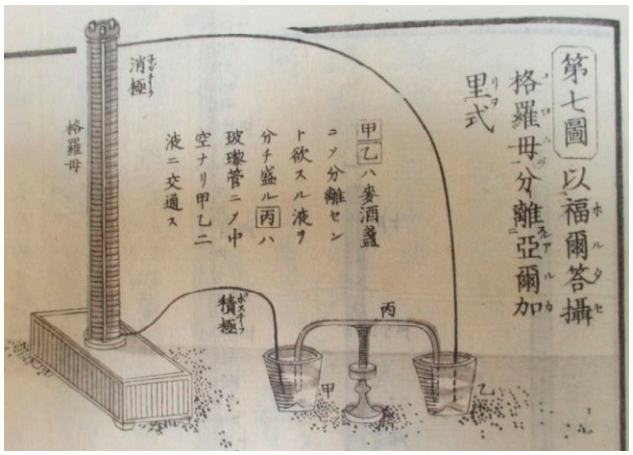

Figure 6. Youan's Volta pile sketch and explanation. SK book 1, vol. 3 Figure 7. Photo: Y. Schley.

produce, the nature of saline or other ingredients.[17]

Youan's translation, in the external, seventh book first volume, chapter 1-2 on mineral spring:

> Water is not a pure thing. In a popular way, it can be said that water is pure, but, by using a reagent it can be seen that it is not pure. From under the sky water goes down to the earth and penetrates. Then [coming out] from the earth a spring is formed, in it a part of mineral matter, naturally consisting of not a small amount of impurities.[18]

It is clear that Youan follows Henry in this section, even though it is a translation from Ypey's Dutch translation of Trommsdorff's German translation of William Henry's English text.

Mr. Volta Column (See Figure 6)

Youan describes the finding in 1791 by Galvani "by chance how electric power is generated and explained this fact to encourage junior. Volta (Alexandro) in the city made a column like a tower to generate electricity by piling up many metal plates whose form (is) oval." Youan gave a detailed description and drawing of its construction:[19]

> Volta's column is an unusual device of modern invention. The following is the construction: zinc (or tin) is casted into the oval form whose size is that of Dardel (the diameter is about one sun) and a little thicker than the Dardel.[20] Next, silver (or copper) is used to make the same size of oval and moreover felt (or thick paper) is used to make the size of oval. Then three kinds of 30 to 50 plates are piled up in order to make a column. The first is silver plate, the second is zinc and the last is felt dipped in condensed salty water and squeezed after it. Piling 20 to 30 sets, the last top plate is zinc plate [in the original paper's misprint stated that the last is silver plate]. On the bottom silver plate a strip of tin or lead is pierced which works as a contact to outside. If a tester touches the strip with his finger soaked in condensed salty water and another finger touches the top plate of zinc, he will get a shock in both arms. This shock strength is dependent upon the number of piled plates. (According to one theory, when the silver plate is used, salt water is effective, while for the copper plate, ammonium chloride solution is effective).

Youan added comment to his text:

> The pole of the power generated from the silver plate is named cathode (*negatief, ontkennende pool*), and the power pole from zinc is named anode (*positief, stellige pool*). These two poles are different from each other as follows. ○ Anode is signed as +. The taste on tongue is alkali; it changes the color of *akana* solution to red.[21]

Nitrogen oxides compounds

In the following text Youan tells about Cavendish's discovery by citing Smallenburg's book. Youan invented terms for the degrees of oxidation in Japanese.

> Chemical combination of nitrogen and oxygen forms nitric acid. Nitric acid is formed from the combination of nitrogen and oxygen. Into nitrogen gas oxygen gas is mixed, electric spark is passed through, and nitric acid is formed. …According to "Smallenburg's Chemistry," in 1784, an Englishman, Cavendish, mentioned nitric acid composition for the first time. …According to several French and Dutch scholars, in nitrogen and oxygen compounds there are four grades.[22]

Foreign spas in Youan's Seimi Kaiso

Youan mentioned five springs in Bohemia, these include:

> Bernard Spring – there is a big building – it is called hospital; Mill Spring, since 1711. Temp. 138 degrees Fahrenheit [ca. 59° Celsius]." Youan mentioned the spring's therapeutic effect. "New Spring since 1748. Temp. 145 deg. Fahrenheit. It is attributed medical curative effects; Hot Spring - since 1725. Temp. 165 deg. Fahrenheit. Attributed medical benefits; Telesia Spring nearby. Temp. 135 deg. Fahrenheit: 'Many women patients are bathing.'
>
> The above 5 Springs compositions is about the same, including: 硫酸ソーダ: Na_2SO_4 sodium sulfate 24-46

grain; 塩酸ソーダ: NaCl sodium chloride 5-6 grain; 炭酸ソーダ: Na_2CO_3 sodium carbonate 10-15 grain; 炭酸カルキ: $CaCO_3$ containing CaO calcium carbonate – lime 1-4 grain; 酸化鉄: Mainly Fe_2O_3 iron oxide 0.02 grain; 炭酸ガス: CO_2 carbon dioxide gas ~ 5-30 cm^3; 硫酸苦土泉: $MgSO_4$.[23] 1 grain ~ 0.02 gram. Hot springs contain magnesium sulfate and iron oxide. The taste is bitter. Bitter taste comes from calcium and magnesium sulfate and the color from iron oxide.

Analysis of chemical ingredients of hot springs in Japan

Japan is famous for its abundance of hot springs and the tradition of hot springs bathing. Udagawa Youan studied the chemistry of the water in hot springs. A thorough study of Udagawa Youan research on chemical ingredients in hot spring was published by Osawa Masumi (1932-). Osawa studied Von Siebold's books and Bürger research on mineral contents of hot springs in Japan.[24] Some of von Siebold chemical collections are stored in the Museum of Ethnology in Leiden (*Museum Volkenkunde*). The reagents used by Youan are stored at Waseda University Archive. According to Osawa, "Chemical analysis of mineral spring water was first carried out by P.F. von Siebold (1796-1866) and H. Bürger (1806 -1858), medical officers of the Dutch East India Company (VOC) in Nagasaki, for several samples from Kyushu, southwest Japan in 1820s." When they went to Edo (now Tokyo) in 1826, they met Udagawa Youan (1798-1846), at Nagasakiya inn located in Hongokucho, Edo city several times during the 3rd day of March to the 12th day of April (lunar calendar).[25] They probably discussed mineral springs among other topics like Japanese plants. A lot of chemical reagents and equipment were carried to Japan when Siebold came to Nagasaki in 1823. Siebold and Bürger probably brought them to Edo. Then, from 1828, Udagawa started his chemical study of mineral springs from a wide area of Japan."[26] Osawa cites a summary of minerals found by Youan in Suwa, Shinshu (today Nagano prefecture) in 1829 (*Bunsei* 11) as written in a draft kept at Osaka Takeda Science Foundation Library. For example: ｽﾜｰﾌﾙｼﾞｭｰﾙ *Zwavelzuur,* 硫酸 *ryuusan*, sulphuric acid.[27]

4. COINING CHEMISTRY VOCABULARY AND THE DEVELOPMENT OF CHEMISTRY LANGUAGE IN JAPAN

Youan's terms, their original Dutch and their survival

Table 1 is composed of three contributions: Sakaguchi Masao listed 58 Japanese terms, as they were used in 1975, and he put in parentheses Youan's terms. Sakaguchi's Japanese list was followed by a list of the same terms in Dutch (1). The list in Japanese was previously published by Tanaka M. in 1964 (2).[28] English translation is added by the current author (3). It shows Udagawa Youan inventions of various terms for chemistry tools and processes. For most of the words Youan combined two characters that should transfer the meaning of the Dutch term into Japanese. Some words like no. 48 *cork* and no. 51 *retort* were written by Youan in kanji as *ateji*, phonetic pronunciation.

Several of the terms that were formed by two kanji combination were preserved and are still used today, e.g., 結晶 crystal. Others have been changed, either by one, e.g. 燃焼 combustion or both kanji letters, e.g., 融点 melting point. It may be said that Youan understood the meaning of the terms that he was translating and chose the appropriate kanji for them. Those new terms added to their practical use in chemical processing since the nineteenth century until today. For example: entry no. 40: 飽和 *houwa*, saturation, is formed by 飽 tired of, satiate, and 和 that has several meanings: harmony, Japanese style, peace, Japan. The same term 飽和 is used today for saturated fatty acid, as in 飽和 脂肪酸 *houwa shibousan*. Thus, contemporary scientists find it appropriate to use Youan's kanji combination for the Dutch term *verzadiging*, saturation. Another example, no. 41: Dutch: *opheffing* 昇華 *Shouka*, meaning sublimation: 昇- rise up 華 has several meanings: splendor, flower, gorgeous. This term is used today for transfer of matter directly from solid to gas. It is also used for sublimation in psychology.[29]

In his chapter on "Youan the Linguistic," Takahashi Terukazu (1944-) showed several kanji letters combinations that Youan chose in order to use phonetically. E.g., *an* 諳安, *ba* 抜婆、*ta* 太. [30] He used them for no. 51 in the table, 列篤爾多レトルト for retort; for writing the Western names shown in Fig. 4, e.g. 布廉吉 フレンキ佛如 Plenck; and for writing names of foreign countries, e.g. 波尓杜瓦尓 ポルトガル for Portugal. Other terms have a combination of katakana and kanji, like *litmus paper* ラッカムース紙. The pronunciation of no. 48 *Cork* コルク and no. 49 *beaker* ビーカー in today's reading is somewhat different than Youan's, possibly due to change in pronunciation during the years.[31]

The birth of the term 元素 *genso,* element. In 1834 Youan published his book *Shokugaku Keigen* 植学啓原 (Introduction to Physical Science. Principle of Botany). In its third, last volume, he addressed plant biochemistry; it became a textbook for natural sciences. In *Shokugaku Keigen* third volume there is the following exposition for the first time: Air, water, oil, salt. He used the

Table 1. Current and Youan's chemistry terms, Dutch terms he studied and their English translation.

	Dutch (1)	Recent Japanese (1,2)	Udagawa Youan (1)	English(3)
1	wet	法則	法則	law
2	eigenschap	性質	稟性	property
3	ontbinding	溶解､分解	分離	Dissolution, separation
4	Scheikundige probeermiddelen (reagentia)	化学者 試みの手段 (試薬)	試薬	chemist testing means (reagents)
5	droogeweg	乾式法	燥道の法	dry way method
6	onbewerktuigde ligchamen	無機物	無機性体	inorganic substance
7	bewerktuigde ligchamen	有機物	機性体	organic substance
8	verbranding	燃焼	熱焼	combusion
9	bestanddeel	成分	成分	component
10	volumen uitgebreidheid	容積 示量	容積	volume extensiveness
11	gewigt	重量	秤量	weight
12	eigendommelijke zwaarte	比重	異類重	specific gravity
13	gaz	ガス	瓦斯	gas
14	damp	蒸気	蒸気	vapor
15	vaste lichaamen	固体	凝体	solid
16	vloeibaare lichaamen	液体	流体	liquid
17	drukking	圧力	圧力	pressure
18	temperatuur	温度	温度	temperature
19	kooking opbruisching	沸騰 ドレッシング	沸騰	boiling dressing
20	het punt van kooking	沸点	沸度	boiling point
21	meltpunt	融点	熔度	melting point
22	uitzetting	膨張	廓張	expansion
23	vermeerdering van warmte	発熱	熱起	fever
24	luchtledige	真空	無気	vacuum
25	electriek, electriiteit	電気	越列気　エレキ	electric, electricity
26	stellige (positief) pool	陽極	積極	anode (positive) pole
27	ontkennende (negatief) pool	陰極	消極	cathode (negative) pole
28	kooken	煮沸	煮沸	boiling
29	vervliegen	揮発	揮散	volatilization
30	uitdamping	蒸発	蒸散	evaporation
31	droogheid, uitdroogen	乾 涸	乾固	dry up
32	overhauling	蒸溜	蒸餾	distillation
33	drooge overhauling	乾 溜	乾餾	dry distillation
34	schudding	振盪	振蕩	shock
35	beweging	攪拌	攪擾	stir
36	kristal	結晶	結晶	crystal
37	vervloeien in de lucht	潮解	潮解	deliquescence
38	oplossing	溶液	溶液	solution
39	filtreeren	濾過	濾過	filtration
40	verzadiging	飽和	飽和	saturation
41	opheffing	昇華	昇華	sublimation
42	nederplofsel benzinkzel	沈降 沈殿	澱	sedimentation precipitation
43	toestel	装置	装置	device
44	lakmoespapier	リトルマス紙	勒法母斯ラッカマース紙	litmus paper

Table 1. (Continued).

	Dutch (1)	Recent Japanese (1,2)	Udagawa Youan (1)	English(3)
45	curumapapier	クルクム紙	姜黄紙	turmeric paper
46	smelt-kroes	坩堝	坩堝	crucible
47	blaaspijp	吹管	吹管	Blowpipe
48	kurk	コルク	鳩爾古 キュルく	cork
49	bekerglas	ビーカー	玻黎ベーケル	beaker
50	flesschen	フラスコ	フラスコ	flask
51	retort kromhals	レトルト	列篤爾多レトルト	retort
52	glaspijp	ガラス管	玻黎筥	Glass tube
53	schaal	目盛	度目どめ	scale
54	kraan	蛇口	回銓 かいせん	tap
55	luchtledige klok	真空計	排気鍾はいきしょう	Vacuum clock
56	eudiometer	水「ガス」電量計	欧実阿墨多爾 ユーヂオメートル	eudiometer
57	thermometer	温度計	験温器	thermometer
58	calorimeter	熱量計	験熱器　カロリメートル	calorimeter

Table 2. Choices of kanji for element by Udagawa Youan and other scholars.

Udagawa Youan 宇田川榕庵	Hoashi Banri 帆足万里	Takano Choei 高野長英	Aochi Rinso 青地林宗
元素 *genso* element	原質	造質/原質	原質
酸素 *sanso* oxygen	酸質	酸質	酸質
窒素 Suffocating element- nitrogen 殺素 Lethal element	塞質	窒質 suffocating matter	窒気 suffocating gas
水素 *suiso* hydrogen	水質	水質	水質
炭素 *tanso* carbon	炭質	炭質	煤質

character so -素 for several elements: Oxygen, *zuurstof* is *sanso* 酸素; Nitrogen, *stikstof* is *chisso* 窒素; Hydrogen, *waterstof* is *suiso* 水素; Carbon, *koolstof* is *tanso* 炭素.

Genso 元素 is translated from *grondstof*. 元 *grond* meaning basis, 素 is equivalent to 物質 *busshitsu,* substance, matter*, stof in Dutch.*[32]

Youan used those terms for the first time in his botany book in 1834. However, other Japanese Dutch scholars have used the ending term [~質] *shitsu*, meaning substance, matter. Aochi Rinso 青地林宗 in his book *Kikai Kanran*, Overall View of the Atmosphere, of 1827; Takano Choei 高野長英 in his book 西説医原枢要*seisetsu igen suoyou,* Western Explanation of the Theory of Physiology, published in 1832; and Hoashi Banri 青地林宗 in 窮理通 *kyuuritsuu*, Generalities of Physics, (ca. 1836).[33] A comparison of the various kanji characters choices is shown in table 2.

Several other terms with different kanji were used by scholars. For example, *Caloric: Onshitsu* 温質 *Matter of warmth.* Youan*: Danso* 煖素. For nitrogen, Youan tried two different kanji combinations: 殺素 lethal element and *Chisso* 窒素 that is the term used to this day.[34]

Sugawara Kunika studied Misaki Shosuke (1847-1873) translation of Fresenius. Misaki, used terms coined by Youan, but no citation of Youan is shown.[35] Those words like 硫酸, 硝酸 for *Zwavelzuur* or *Salpeterzuur* should be from Youan. But, those words like 能溶薬, 硫化炭精, or 造塩質属 for *enkelvoudige oplossingsmiddelen*, *Zwavelkoolstof*, or *haloiden*, are probably not from Youan.[36]

Further evolution of chemistry language in Japan

Tsukahara Togo in the Introduction to his Ph.D. dissertation pointed out the influence of *Seimi Kaiso* on writing new chemistry books in Japan immediately after its publication and even fifty years later. That was in spite of the developments in chemistry in the world during those years, the second half of the nineteenth century. Tsukahara mentioned that in the curriculum of *Kaiseijo*, a governmental institute for Western learning founded in 1866, *Seimi Kaiso* was designated as a textbook for chemistry. Tsukahara observed that "…it is

righteous to assume that *Seimi Kaiso* paved the way for the introduction of Western chemistry in Japan, which was a prerequisite and indispensable condition for the development of chemical industry." He assured that "the creation of a new vocabulary was by all means the most essential part of the introduction of Western science in Japan."[37]

Kaji Masanori (1956-2016) mentioned those who followed Youan's chemistry book *Seimi Kaiso*. Among those was Kawamoto Kōmin 川本幸民 (1810-1871), a teacher of chemistry at the *Bansho Shirabesho,* School of Western Learning, who translated a number of chemistry textbooks, such as *Kagakushi Shinsho,* A New Book of Chemistry. In that book Kawamoto wrote for the first time concepts that were not in *Seimi Kaiso* like: *genshi* 原子 atom, *bunshi* 分子 molecule. In addition, topics like *tampaku* 蛋白 protein, *budoutou* ブドウ糖, grapes sugar, glucose, *nyouso* 尿素 urea, and the like are seen in *Kagakushi Shinsho* for the first time. Kawamoto wrote a text book on Dalton's atomic theory.[38]

The topic of the vocabulary and teaching language of chemistry remains relevant in Japan. First generation of Japanese chemistry teachers after 1868 Meiji Restoration studied in Europe and taught chemistry in English (or German?). As Kikuchi Yoshiyuki described "Sakurai [jōji] gave at least some of his lectures in English. His lectures on chemical philosophy in 1882-1883 at Tokyo University were in English… However…, teaching in Japanese became the norm by 1884 throughout Tokyo University and its preparatory schools as the number of foreign teachers decreased."[39]

5. EUROPEAN KNOWLEDGE EXCHANGE VS. JAPANESE ISOLATION

Many connections and extensive knowledge transfer existed between scholars in Europe: by personal correspondence, by scholars visiting scholars in other countries and by circulations of scientific journals. This is in comparison with the Japanese who were secluded from most of the world with almost no possibility to exchange knowledge with others out of Japan, except China, and restricted conditions for exchanging knowledge within Japan.

The political situation in Japan, the strict observance and surveillance of the citizens by the Bakufu authorities, including watching scholars of Dutch Studies, should be taken into consideration. E.g., watching the books that were allowed to enter the country and to be studied, and forbidding transfer of knowledge to lower rank people.[40] Moreover, as Goodman explained "…some orthodox Cunfucianists held to their belief that the Westerners would make use of Christianity to invade Japan. To the extent that all Western scholarship was considered as a tool of the religion of Christ, the work of the *Rangakusha* was subjected to the oppressive scrutiny."[41]

Fear of the persecution of scholars of Dutch Studies was also expressed by Fukuzawa Yukichi (1835-1901) in his autobiography. It is cited by Blacker from his memories that "had it been safe to do so he would certainly have taken western learning beyond the stage of scientific techniques and advocated it as a weapon against bullying feudal officials as well as against bullying foreigners."[42]

Interestingly, Fukuzawa told about running chemistry experiments with other students during his studies in the *Tekijuku* School in Osaka, directed by Ogata Kōan, a physician of Western medicine, in 1854-1855.[43] Fukuzawa decided to start learning English after he realized that his investment in studying Dutch was not useful when he wanted to speak and understand the American sailors of Commodore Perry's ships.

In Europe at the same time, a wide and intensive exchange of knowledge existed in the seventieth through the nineteenth centuries between scholars; by correspondence, reading and translating articles, as well as personal visits, e.g., Berzelius visit in France in summer 1818,[44] Berzelius visit with Davy in London in 1812 and their correspondence 1808-1813, as well as Berzelius correspondence with Wöhler, Berthollet, Mulder, Mitcherlich and many others.[45] William Henry in "The Elements of Experimental of Chemistry", in his examples and discussion in the chapter on analyzing water he mentioned Dr. Wollaston, Mr. Watt and Berzelius.[46] French, English, German, Swedish and Italian scientists were exchanging scientific knowledge, discussing information, arguing about their philosophical ideas and the interpretation of the results of experiments.

In contrast, Japanese scholars were isolated from the Western world and could hardly get any information from Europe. In 1826 and until 1830 Youan received some help from von Siebold and Bürger in botany, plant drawings, and hot spring water analysis. But von Siebold was not in Japan while Youan wrote *Seimi Kaiso* since 1836. In his letter to his friend and disciple Ito Keisuke (1803-1901) Youan complained that he could not meet foreigners in Edo and could not get chemistry books from them.[47]

Two events show Youan defending himself from the ruling authorities. After what is called the Siebold Incident in 1829, the work of the translation office was stopped by the Bakufu authority. On March 25, 1829, Udagawa Genshin, Youan and other members of the translation office wrote a letter to the authorities, saying

that they did not have any connection with that affair and asking to let the office continue its important translation work.[48]

We learn about a second event concerning Youan under such a prevailing socio-political, anti-Western spirit and anti-Christianity atmosphere. Takahashi Terukazu raised a question – "Was Youan Christian?" and presented a document that was written in order to remove suspicions against Youan who was involved in studies of Western books. The document dated 1834 is preserved in Waseda University Library, shows a declaration by the Head Priest of a Buddhist temple in Asakusa, Edo, concerning Udagawa Youan belonging to his Buddhist temple, and that Youan did not become a follower of Christ *Yasu* (Jesus).The priest declaration states that Youan's writing room was named 菩薩楼 *bosatsurou*, Bodhisattva Room, after a Buddhist Scripture, the Heart Sutra 般若心経 *hannyashingyou*. Youan attached the phrases from the Heart Sutra on the wall of his writing room.[49] Udagawa Youan did not know much about Christianity, he studied Western science without leaving his religious faith. Concerning Youan's religion, Goodman concluded that "… despite all his remarkable credentials as a *Rangakusha*, Youan was, like his father and Otsuki Gentaku before him, a committed Confucian scholar, devoted first and foremost to the Classical Chinese intellectual heritage of Japan."[50] In light of the continuing surveillance the achievements of Udagawa Youan, and indeed his colleagues, are even more impressive.

6. FURTHER CHEMISTRY STUDIES FROM THE WEST

Concerning the Japanese Dutch scholars, Tsukahara observed that "It is an over simplification to say that the Japanese have only copied Western sciences and exploited its practical parts. Philosophical discussions and practical demand were interrelated; they were interwoven into a new pattern of theory and practice, slightly different from that of the West. Likewise, it would also be a distortion to suppose that this interaction involved nothing more than the relationship between "pure" and "applied" sciences. Scientific theory and technical practice were merged in *Rangaku*. This tradition was a remarkable feature of science in Japan."[51]

By the middle of the 1850s the Japanese had both skillful capacity for craft production and basic scientific knowledge translated and adapted from the West. A change of attitude started after the arrival of Commodore Perry from America by the "Black Ships" in 1853 and again in 1854. One of its results was the opening of several Japanese ports to foreign ships. In 1868 the Meiji Restoration rejected the long feudal rule of Tokugawa and brought the Emperor back into power. Confronted with the American ships, cannons and other demonstration, the Japanese realized that they are not as advanced as they have believed, actually lagging behind the Western knowledge for large ships building, for the constructions of railroads and trains and manufacturing weapon like cannons. This realization was concluded in the decision to learn technology from the West, while keeping the Japanese spirit. Nevertheless, the educator Fukuzawa Yukichi explained that studying just the surface of technology is not enough if one wants to be able to further develop things by oneself.

In order to make progress in chemistry science and technology the Japanese invited foreign teachers to come and teach in Japan. Late 1860s to early 1870s, two foreign chemistry teachers were the American William Griffis (1843-1928) and the Dutch Konraad Wolter Gratama (1831-1888).

Early publication of chemistry textbooks in Japan since the 1870s included the translation of the chemistry lectures by American William Griffis (1843-1928) in Fukui. In a letter to Philadelphia to his sister Margaret Clark Griffis on June 25th 1871 he wrote: "In chemistry, I have carried out two classes through oxygen, nitrogen, hydrogen, sulfur, chlorine and carbon and their compounds." These lessons were translated by his students into Japanese and circulated among them. In a letter of July 15th 1871 he asked his sister to send him a copy of Roscoe's *Chemistry*, latest American edition. Teaching chemistry from Roscoe's book was later spread in Japan.[52] Roscoe's book was published in the same year, so Griffis could teach from an advanced chemistry book of his time [53]. Japanese students in the laboratory of Henry Enfield Roscoe (1833-1915) in Manchester translated his 1866 chemistry book into Japanese. Ichikawa Seizaburo's (*alas* Morisaburo) translation "chemistry entry book for elementary school" was published by the Ministry of Education 1873. Griffis moved to Tokyo after eight months in Fukui. His students in Tokyo became the first generation of Meiji chemists.

Another translation was of the chemistry course taught by the Dutch Konraad Wolter Gratama (1831-1888) in *seimikyoku*, the Chemistry School in Osaka specifically built according to his design. It was built for instructing technicians, methods to separate metals from the ores excavated in mines that included copper, silver and gold. Gratama used reagents, analytical tools and reference books that he brought with him by ship in nearly two hundred crates to Nagasaki in 1866. Gratama chemistry lectures were translated into Japanese by Misaki Shosuke 三崎肅輔 (1847-1873) into *seimikyoku kaiko*

no setsu, Chemistry Theory Course, 1869. The translated books were further circulated and studied in Japan.[54]

In the conclusion of his article Kaji observed: "The discovery of the periodic law between 1869 and 1871 and its dissemination in the 1880s coincided with the institutionalization of chemistry in Japan. This factor helped make the appreciation of the periodic system as a basis for chemistry in Japan easier. Most of the first generation of Japanese chemistry professors accepted the periodic law as one of the recent developments in chemistry in Europe without much doubt.[55]

The department of chemistry was founded in the governmental Institute for Western Learning, *kaiseijo*, in 1866. It became a Department of Chemistry of Tokyo University in 1877. For the role of foreign chemistry teachers at Tokyo University see for example Kikuchi Yoshiyuki's book.[56]

The Chemical Society of Japan (CSJ) was founded in 1878 "by approximately twenty motivated and enthusiastic young scholars wishing to advance research in chemistry."[57] They formed a committee to assemble chemistry dictionary, it worked for more than ten years.[58] The first English-Japanese chemistry dictionary that was the result of the work of the (Tokyo) Chemical Society of Japan was published in 1891. It presents in ABC order chemical names, experimental tools, processes etc., and contains Japanese terms in kanji, katakana, and their combinations. It reflects the development of chemical theory and the change of the dominant foreign language from Dutch to English.[59]

Detailed description of the current Japanese rules of naming chemistry compounds can be found in the *Japanese-English Chemical Dictionary* edited by Markus Gewehr, 2007.[60]

SUMMARY

This work presents Udagawa Youan pioneering studies of chemistry from Western books. He studied botany first and then chemistry and wrote several books before writing his larger book *Seimi Kaiso*, Introduction to Chemistry. He translated chemistry from Western scientific books in Dutch that are presented in this study. For the translation Youan coined new terms in Japanese. He could choose appropriate Chinese-Japanese characters to transfer the meaning of words from Dutch to Japanese, trying to shift the new terms from memories of the prevailing Confucian view of the world. The difficulty in moving from the Eastern philosophical thought to the Western is partly discussed. The Confucian traditional priesthood objection to introduction of foreign ideas contributed to obstacles faced by Youan and other *rangakusha*. Another difficulty pointed at was the objection of the ruling Bakufu to wide spread of Western knowledge. Still, Udagawa Youan's successful pioneering of chemistry translation and terminology can be considered as a milestone in Japanese modernization.

Chemistry studies and practice continued after Youan, using some of the vocabulary he invented. Teaching materials of foreign teachers in Japan, mentioned above are Griffis and Gratama, were translated into Japanese. Roscoe's book was also translated by his chemistry students in England. Following Meiji Restoration there was further progress in scientific studies, and the establishment of Tokyo University and other national Universities led to the creation of a successful Japanese academy and a prosperous chemical industry.

ACKNOWLEDGEMENTS

This research started as a grant recipient from Japan Foundation that let me spend six months at Tokyo Institute of Technology, at the unit of Prof. Kaji Masanori. He introduced me to the name of Udagawa Youan, and continued guiding me until the very day of his hospitalization on 12 July 2016, just before his untimely death. He is very much missed. My research continued during one year fellowship as a visiting scholar at Nichibunken, International Research Center for Japanese Studies in Kyoto, April 2009-March 2010. The devoted instruction of Prof. Frederik Cryns enabled me to continue the research after returning to my country and our discussions during annual short visits to Japan. I thank the scholars who helped me in various steps of this work for their advice and information: Prof. Sato Shin, my first chemistry teacher in Japan, Prof. Dōke Tatsumasa, Prof. Azuma Toru, Prof. Furukawa Yasu, Prof. Ochiai Hirofumi, Prof. Osawa Masumi, Mr. Uchida Masao, Prof. Ohmichi Naoto, Prof. Kato Nobushige, Dr. Yakup Bektas, Dr. Karl Grandin, Prof. Shimon Vega, Dr. Jochanan de Graaf, and Dr. Neomy Soffer. Colleagues of the Japanese Society for the History of Chemistry in Japan are deeply acknowledged. My gratitude is extended to the librarians at Nichibunken and to Ms. Chaya Meier Herr at the Edelstein Collection for the History of Science at the National Library of Israel in Jerusalem.

NOTES

1. T. Dōke, **1973** p. 99. Siderer, **2017**, p. 224.
2. Dōke, **1973** p. 105; Takahashi **2002** p. 172. Youan had access to maps of the world, at least one, a

map called "*shinsensokaizenzu* 新鐫総界全図"New engraved whole world map", 1809 (Ref. stored in the Waseda University Library, call mark is "bunko 08 c0995 (文庫08 c0995); Takahashi Terukazu p. 172. Takahashi mentioned the map drawn by the Italian Mateo Richi in China, *Great Map of Ten Thousand Countries*, in the beginning of 17th century and its revised map by Takahashi Kageyasu's *shinteibankokuzu* 新訂万国図 *shinteibankokuzu* New revision of Ten Thousand countries 1810, that Youan received.

3. Chomel Encyclopedia.
4. Mac Lean **1974**, p. 9.
5. T. Shiba **2010;** T. Azuma**, 2013;** T. Azuma **2017; Y.** Siderer **2017**.
6. K. Sugawara, **1987** p. 29. In Japanese (J), English abstract.
7. On *Seimi Kaiso* first page of each book there is a warning against forgery: "Our shop was opened outside the Asakusa castle gate, at the east of Kikayamachi-Asa(?), and it possesses all the books written by Master Udagawa, over a span of three generation, which our shop assembled entirely, made books and published. All our favorite wise customers! We recommend you surely to confirm the seal authenticity of each volume of the books. If there is a seal, the book is authenticated. If there is a fake seal, such book is a pirate edition. Sincerely yours, Owner of this bookstore, Seireikaku." Thanks to Prof. Ohmichi Naoto for this translation.
8. SK book 1, preface; SKR p. 11.
9. Tanaka Minoru 田中実 Ed., The authors of the modern revision and comments on Youan's *Seimi Kaiso* are: Books 1, 2,3 and 7: Hayashi Yoshishige 林良重; book 4: Kurokui Seiji 黒杭清治; book 5: Kusuyama Kazuo 楠山和雄; book 6: Kanazawa Shouji 金沢昭二.
10. M. Tanaka, M. Sakaguchi, T. Dōke, T. Kikuchi **1975**.
11. Copies of old prints of *Seimi Kaiso* books nos. 1-6 this author received with thanks from Prof. Osawa Masumi.
12. Tsukahara, **1993**, Hoffmann p. 319 and Serrurier p. 325.
13. The list follows researches on: *Seimi Kaiso* Rewritten **1975** shortened SKR; Sakaguchi Masao article in SKA **1975**; Tsukahara Togo **1993**; Azuma Toru **2006-2020**; Osawa Masumi since 2006; Miyashita Saburo **1997,** and references cited in those articles.
14. SKR index pp. 568-540.
15. Tsukahara, **1993**, p. 148.
16. M. Tanaka, **1975**, in SKA p. 104.
17. Henry, Epitome of Chemistry, **1808**, p. 413.
18. SK external book, vol. 1 ch. 2; SKR p. 474.
19. SK book drawing on last pages, Figure 7; SKR p. 78.
20. Currency name: A *daalder* is a silver coin which was first minted around 1500 in Joachimsthal (Tyrol), hence the name 'Joachimstaler' which later became 'taler' or 'daalder'.
21. SK book 1, vol. 2, ch. 50, Mr. Volta Column; SKR pp. 54. Thanks to Prof. S. Sato for the translation.
22. SK book 2 ch. 101; SKR, **1975**, p. 118, comment 10 p. 161 on nitrogen oxide compounds.
23. *Seimi Kaiso* External book vol. 3; SKR pp. 519-520.
24. Osawa in Onsen **2018** winter issue**.**
25. Thanks to Prof. Kato Nobushige for the dates of Siebold in Edo. E-mail dated 1.10.2019.
26. Osawa, **2009,** p. 84.
27. Osawa, in Onsen **2019** spring issue, p. 35.
28. Sakaguchi SKA **1975**, p.57; Tanaka **1964**.
29. *Denshi Jisho* 22.5.2020. Sped Terra Shogakukan Professional English Dictionary **2004**, p. 1623.
30. Takahashi, **2002**, p. 174.
31. Tsujimura, **2007** on Language Variation. p. 422. Frellesvig Bjarke, **2011**.
32. After Takahashi **2002**, p. 157
33. Goodman, **2014** (2000), authors' names and books: Takano Choei p. 202, Hoashi Banri p. 113, Aochi Rinso 153.
34. Shimao, **1972**, p. 317, p. 319.
35. Sugawara, **1984**.
36. Uchida, email correspondence 15.8.2020.
37. Tsukahara, **1993**, p. 1.
38. Kaji, **2015**, p. 286; *Encyclopedic Dictionary* **2017**, p. 168 (J); *Invitation to Chemistry History* **2019** p. 242 (J).
39. Y. Kikuchi, **2013**, p. 134; Appendix p. 175 and reference cited there.
40. Marie-Christine Skuncke, **2014**, p. 110.
41. Goodman, **2014**, p. 199.
42. Blacker, **1969**, p. 25.
43. The Autobiography of Yukichi Fukuzawa **1901,** p. 90.
44. Carl Gustaf Bernhard, *Avec Berzelius en France Parmi ses Genies et ses Volcans Eteints*. Pergamon Press 1985 (French 1989).
45. Jaqueline Reynolds and Charles Tanford, *Science,* Nature's Robots: A History of Proteins **2003**.
46. William Henry. **1808,** p. 415.
47. Dōke, **1973**, p. 109; Dōke, SKA **1975**, p. 84.
48. Goodman, **2014,** p. 187.
49. Takahashi, **2002**, p. 140.
50. Goodman, **2014**, p. 139; F. Cryns, personal discussion.
51. Tsukahara, **1993**, p. 3. Kaji, **2015**, p. 284, p. 286. Kikuchi, **2013**. pp. 97-100.
52. Y. Siderer. Presentation at the 21st International Society for the Philosophy of Chemistry (ISPC) conference, 5 July Paris **2017**. Submitted 2017.

53. Kurahara, **1995,** p. 1**.**
54. Misaki, **1869**; Shihara and McAbee, **1988** p. , Sugawara, **1984**, p. 20. Uchida et al. **1990?**, p. 247.
55. Kaji, **2015**, p. 299.
56. Kikuchi **201**3, ch.2, p. 27. Kaji, **2015**, p. 289. Furukawa **2019**.
57. Common Knowledge. The Chemical Society of Japan, president Kobayashi Yoshimitsu **2019**.
58. Takata Seiji, **1995**, (J).
59. M. Uchida, **2014**, personal communication.
60. Japanese-English Chemical Dictionary: Including a Guide to Japanese Patents and Scientific Literature, Markus Gewehr (Editor). ISBN: 978-3-527-31293-1 November **2007**.
61. Tsukahara, **1993**, p. 268, C1.
62. Azuma. *Kagakushi*, The Journal of the Japanese Society for the History of Chemistry **2006,** vol. 33, No. 3, 129 (1)**.**
63. SK book 6, vol. 16, ch. 266; SKR p. 407; SK Book 6 vol. 17 ch. 279; SKR p. 442.
64. SK book 3 vol. 7 ch. 144; SKR, **1975**, p. 187.
65. Tsukahara **1993**, C.2 p. 268 and C.9 p. 272.
66. SK Book 2, vol. 4, ch. 84, SKR p. 103.
67. SK book 3, vol. 9, ch. 167; SKR p. 220. See Tsukahara C.9. p.272 for further discussion.
68. After 元素の秘密がわかる本, Gakken Publishing **2015**, p. 158.
69. Tsukahara, **1993**, p. 268.
70. Tsukahara, **1993**, C.5, p. 270.
71. Lavoisier, **1789**, French p. 200. Dutch **1800** p. 198.
72. Miyashita, **1997** no. 7, p. 74.
73. SKR p.13. SK first book preface p. 5.
74. Azuma, in *Kagakushi* journal **2013**, vol. 40, no. 4 p. 189 (19). *Kagakushi* journal **2014**, vol. 41, no. 1 p. 1 (1).
75. SK preface p. 7; SKR **1975**, p. 14.
76. SKA, **1975**, p. 24. Tsukahara, **1993**, p. 280. Mac Lean **1974**, p. 45, p. 50.
77. SK book 1, vol. 1 p. 7; SKR, **1975**, p. 14; Tsukahara, **1993**, p. 272.
78. Tsukahara, **1993**, p. 273; Sakaguchi, SKA, **1975**, p. 25; SK external book vol. 1 ch.10; SKR, **1975**, p. 486.
79. SK book 1, vol. 1, p.7; SKR, **1975**, p. 14; Tsukahara, **1993**, p. 274.
80. SK book 1 vol. 1 p. 7; SKR, **1975**, p. 14 and others; Tsukahara, **1993**, p. 274; Sakaguchi, SKA, **1975**, p. 25.
81. Sakaguchi, **1975,** in 科学史研究 *Kagakushi kenkyuu* **II**, 14, p. 67 (J), English abstract.
82. SK book 1 vol 1, p. 7; SKR **1975**, p. 14.
83. SK preface following p. 7; SK book 1 vol. 1 ch. 13; SKR **1975**, preface p. 15, p. 24; Tsukahara **1993**, p. 277.
84. Tsukahara **1993**, p. 278; SK book 3 vol. 7 ch. 144; SKR, **1975**, p. 187.
85. SK book 4 vol. 11. ch. 185; SKR, **1975**, p. 278; Tsukahara **1993**, p. 278.
86. Miyashita **1997**, no. 159, p. 94.
87. SK Book 1 vol. 1 ch. 18; SKR, **1975**, p. 28; Tsukahara **1993**, p. 279. Sakaguchi **1970** p. 185.
88. SK book 2, vol. 4, ch. 74; SKR, **1975**, p. 94.
89. SK book 2, vol. 4, ch. 79; SKR, **1975**, p. 97.
90. Tsukahara, **1993**, p. 281, C.21.
91. SK Book 1, vol. 3. ch. 51; SKR, **1975**, p. 58.
92. SK book 5, vol. 14, ch. 235; SKR, **1975**, p. 352.
93. Mac Lean **1974**, p. 42 and p. 48.
94. SK book 1, vol. 3 ch. 58 on Kali and Soda; SKR, **1975**, p. 64.
95. Azuma, in *Kagakushi kenkyuu* vol. 44, no. 3, **2017,** p. 117 (5) and 126 (14).
96. Tsukahara, **1993**, ch. VII p. 147.
97. SK book 1, vol. 3, ch. 58; SKR, **1975**, p. 64.
98. SK book 3, vol. 7, ch. 133; SKR, **1975**, p. 175.
99. SK book 3, vol. 8, ch. 155; SKR, **1975**, p. 200.
100. SK book 3, vol. 8, ch. 185; SKR, **1975**, p. 207.
101. SK book 3, vol. 9. ch. 166; SKR, **1975**, p. 219.
102. SK Book 5, vol. 15, ch. 259; SKR, **1975**, p. 386; Tsukahara, **1993**, p. 283.
103. SK book 1, vol. 2, ch. 50; SKR, **1975**, p. 54; Tsukahara, **1993**, p. 284.

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T. Tsukahara, *Affinity and Shinwa Ryoku, Introduction of Western Chemical Concepts in Early Nineteenth-Century Japan*. Ph.D. Thesis. University of Leiden, Holland, J.C. Gieben Publishers, Amsterdam **1993.**

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Udagawa Youan, *Seimi Kaiso.* In Edo: *Suharaya Ihachi* **1837-1847**, in Japanese.

APPENDIX 1.

◯ Kasteleyn, P. J., Descriptive and practical pharmaceutical, economic and physical chemistry 1788.

『葛氏舎密』 *ka shi seimi*, Mr. Ka chemistry. Youan used 葛氏 Mr. *Ka* as name abbreviation when he cited Kasteleyn.

Beschouwende en Werkende pharmaceutische, oeconomische, en natuurkundige Chemie, Tweede 2dln, Amsterdam, 1788.

This book was cited fourteen times in *Seimi Kaiso*, concerning the preparation of chemical reagents, but not theoretical issues. Kasteleyn (1746-1794) represented a group of chemists in the Netherlands at the end of the eighteenth century that was partly against the new doctrine of Lavoisier.[61] Youan favored Lavoisier, but used more of Henry's more practical descriptions.

Azuma Toru (1953-) thoroughly studies Udagawa Youan's chemistry translation by searching articles stored at Kyō-U Archive of Takeda Science Foundation in Osaka and at Waseda University Library in Tokyo. Azuma showed three journals that were edited by Kasteleyn, from which Youan translated chemistry in several of his books. Those books included *metal chemistry*, *Udagawa Chemistry Book*, *Dyeing chemistry*, *Plant chemistry* and *Seimi Kaiso* – introduction to chemistry.[62] In *seimi Kaiso* Youan mentioned Kasteleyn concerning sugar acid, oil extract and oxalic acid.[64] Oxalic acid is mentioned also in an earlier book of Youan, in a citation from Hijmans.[64]

2. ◯ Blumenbach, J. F., The Basic of Physics of the Human Being.

『貌氏人身窮理篇』 *Bushi jinshin Kyurihen* Mr. Bu Basics of the Human being

Groendbeginselen der Natuurkunde van den Mensch. Translated by G. J. Wolff, 1791.

Blumenbach (1752-1840) was one of the founders of comparative anatomy. However, no direct influence on *Seimi Kaiso* can be seen.[65] In the Dutch book the chapters include the human body, liquids in the human body, blood, muscles, respiration and more.

◯ "Three Little Studies". Questions and Answers on Pharmacy.

『三有小學』 *Sanyuu Kogaku*. This book is written under the 9th bullet on SK p. 7, Unknown publication

year. (Figure 5).

Handboek der natuurlijk historie of natuurgeschiedenis. 1802.

The topic of sulfur, its two forms of crystals, e.g. sulfur flowers crystals,[66] and the topic of barium sulfate were written by Youan.[67]

Interestingly, barium was written by Youan as 重土, whereas it is written today using katakana バリウム. It was explained that since barium element is heavier (than the second group in the periodic table), it was called *barys* from Greek language. So Youan used 重い, the kanji for "heavy" to name the barium element.[68]

3. ○ Plenck, J. J.,Physical and chemical description about the liquid in the human body.

『布氏明液論』 *Fu shi mei ekiron 1791*. Mr. Fu treatise on clear liquid.

Natuur-en Scheikundige Verhandeling over de vochten des menschlijken ligchaams1791.[69]

○ Second book by Plenck (SK 5th bullet in Fig. 5): Handbook of Chemistry 1803.

『舎密備要』 *Seimi biyou*

Grondbeginselen der Scheikunde, of oversicht over alle de vakken der Scheikunde, Uithet Lat. Vert. Door J.S. Swaan, Amsterdam, Elwe en Werlingshoff 1803.

Original book was published in Latin in Viena in 1800, titled *Elementa chymiae*.[70]

4. ○ Lavoisier, A. L., Elements of Chemistry, in a new systematic order, containing all the modern discoveries, English translation by R. Kerr, 1790.

『舎密原本』 *Seimi Genpon*. A Principle book for Chemistry.

Grondbeginselen der scheikunde. Utrecht, 1800.

Original French: *Traité Élémentaire de Chemie, présenté dans an ordre nouveau et d'aprés les découvertes modernes*, Paris 1789 Lavoisier, A. L. (1743-1794).[71]

Miyashita mentions three drafts by Youan of the 2nd parts of *Grondbeginselen* that are kept at Takeda Chemical Industries that is Kyō-U Library of Takeda Science Foundation since 1978.[72]

5. ○ See Plenck no. 3.

6. ○ Ypey, Adolph,

『依氏廣義』 *I shi kougi* Mr. I Broad Sense 1804.

Systematisch handboek der beschouwende en werkdadige scheikunde. Amsterdam, 1804-1812.

Ch*emie voor Beginnende Liefhebbers of Aanleiding* 1803.

William Henry's chemistry book – Epitome of Chemistry - was translated into Dutch by A. Ypey. Referred to as CBL for W. Henry Dutch translation: *Chemie, voor beginnende liefhebbers*, *uit het Engelsch*, van J.B. Trommsdorff verm. uitg door A. Ypey, Amsterdam, 1803.

Ypey's Dutch translation was used by Youan while writing *Seimi Kaiso*. In *Seimi Kaiso* preface p. 5 (SKR p. 13) Youan explained the use of books by Henry, Trommsdorff and its Ypey's translations. Youan stated that he mentioned names of only three men but he does not ignore achievements of others.[73] This was the most cited work in *Seimi Kaiso* for theoretical as well as practical topics.

Azuma studied three books in Dutch by Ypey that Youan used. Those are:[74]

- *Systematisch handboek der beschouwende en werkdadige scheikunde*, 5dln, Amsterdam, 1804-1812, in 9 vols. shortened name: SHS
- *Verbeteringen en bijvoegsels tot het systematisch handboek der werkdaadige scheikunde, 3dln*, Amsterdam, 1808-1810
- *Bladwijzer der voornaamste zaken, voorkomende in het systematisch handboek der werkdaadige scheikunde*, Amsterdam, 1812

Azuma showed ten unpublished manuscripts that Youan studied thru Ypey's books on chemistry; Compared Youan's citing SHS, and pointed at the places in Youan's texts corresponding to the places in Ypey's SHS.

7. ○ Niewenhuis, G., Questions and answers on Pharmacy.

『合薬問答』 *Gouyaku Mondou*. Printing date unknown. Questions and answers on Pharmacy.[75]

Bullet no. 19: ○ General Dictionary on art and science for the intellectuals in collaboration with Dutch scientists.

『紐氏韻府』 *Nishi Inpu* Mr. Ni's Dictionary. 1825.

Algemeen woordenboek van kunst en wetenschappen voor den beschaafden stand onder medewerking van een aantal vaderlandsche geleerden bijeenverzameld.

Several copies arrived to Japan by the Dutch ships during 1832 – 1849. Sakaguchi attributed the dictionary to Egbert Buys, Tsukahara discussed other translations and attribution of the dictionary and suggested that Youan used Niewenhuis' dictionary and possibly acquired one.[76]

8. ○ Bernvald, William. van, Medical treatment by electricity 1785.

『越列機療法』 *Ereki Ryoho.1785*. Electricity Treatment.

Over de Geneeskundige Electriciteit, Amsterdam 1785-1789.[77]

9. ○ Bullet 9 is included in Blumenbach no. 2.

10. ○ Hagen, K. G., Pharmacy teaching.

『薬舗指南』 *Yakuho Shinan*. Pharmacy teaching.

Leerboek der apotheker-kunst. Amsterdam, 1807.[78]

It is cited three times in *Seimi Kaiso*, concerning procedures to make ink and tincture.

11. ○ Guiton de Morveau, L. B., The Method of Purifying Atmosphère 1811.

『大気修繕法』 *Taiki Shuuzenhou*.

Verhandelingen over de middelen om der lucht te zuiveren, en de besmetting te voorkomen Leyden, 1802. French Origin: *Traité des moyens de désinfectant l'air* 1801.[79]

12. ○ Trommsdorff, J.B., Experimental finding in Chemistry 1815.

『合薬舎密』 *Gouyaku Seimi*. Medicine chemistry.

Leerboek der artseneimengkundige, proefondervindelijke scheikunde, naar de derde veel verbeterde uitage uit het Hoogduitsche. Amsterdam, translated by N.C. Meppen 1815.

Original German title: *Systematische Handbuch der Pharmacie für angehende Aerzte und Apotheker,* Erfurt, 1792. 2nd. Ed. 1811.

Most frequently cited work in *Seimi Kaiso*. More than 34 times referred to, including theoretical and applied parts.[80] Trommsdorff, J. B. (1770-1837) was also the translator into German of *Epitome of Chemistry* (EOC) by William Henry. (See Ypey no. 6).

13. ○ Ségur, O.,The Sea of Letters on Chemistry.

『舎密翰海』 *Seimi kankai*. 1817. The Sea of Letters on Chemistry.

Brieven over de grondbeginselen der scheikunde: *gewezen leerling bij de polytechnische school*, Rotterdam, 1811.

Original French: *Lettres élémentaires* sur la *chimie* 1803.

Sakaguchi Masao noticed in Udagawa Youan's list of sources for *Seimi Kaiso*, the title *Seimi Kankai* by Octave Ségur. Sakaguchi identified that it is a translation of Octave Ségur's book, written after lessons taught by professors Berthollet, Fourcroy, Chaptal, Guiton de Morveau, etc. Udagawa Youan studied its Dutch translation translated by M.J. Reinhout, a medicine researcher from Leiden, Holland. In Ségur's book, following four chapters with an introduction and explanation about chemistry, the total of thirty two chapters describe topics of crystals of potassium carbonate, ammonium chloride, phosphorus, potassium phosphate, alum, silica, glass, black patina of silver, Iron, mucus, rubber and more.[81]

14. ○ Houte, H. J., Medicine Treaty 1817.

『福烏多薬論』 *Houto Yakuron*. Medicine Treaty by Houte

Handleiding tot de Materies Medica, 1817.

Except for Youan's first list, there is not another citation of this book in *Seimi Kaiso*.[82]

15 ○ Isfording, J. J., Physical handbook for students of medicine.

『理学初歩』 *Rigaku Shoho*.Basics of physical science

Natuurkundig handboek voor leerlingen in de heelen geneeskunde. Amsterdam, Translated by G.J. van Epen 1826.

German original: *Naturlehre für angehende Aertze und Wundärtze, als Einleitung in das Studium der Heilkunde*. Wien 1814.

Tsukahara mentions several translations for this book, but there is only one citation in *Seimi Kaiso*, in a chapter about heat element, Youan adds a note about light element 光素 *kouso*, that he also called *photogeniumu* and further describes the topics of calorique, photon and color.[83]

16. ○ Hijmans, H. S., Outline of General Chemistry

『舎密崖略』 *Seimi Gairyaku* Outline of General Chemistry 1820.

Ontwerp van eene Algemeene scheikunde. Dordrecht, 1820.

Chapter 187 is a discussion on chemical combination of chloride of lime and acids. Specifically, about the affinity between oxalic acid and lime, and boric acid and lime. Tsukahara discusses another book by Hijmans on chemical affinity for which Youan wrote a separate manuscript.[84]

17. ○ Stratingh, E., Chemical Study of Cinchonine and Quinine.

『幾那鹽說』 *Kina ensetsu*. Kina salt theory.

Scheikundige Verhandeling over de Cinchonine en Quinine bevattende eene opgaaf van derzelver verschillende bereidingen, eigenschappen, verbindingen en geneeskundige vermogens, Groningen 1822.[85] An autograph copy kept in Waseda University Library; it is a translation of chapters 1-9 on separation of quinine and 1-4 on its nature.[86]

18. ○ Reinwardt, C. G. C., Treatise on the measurement of the heights of mountains.

『測山説』 *Sokuzan setsu*. Mountain Measuring Theory.

Voorlezingen over de hoogte en vedere natuurlijke gesteldheid van eenige bergen in de Preanger regentschappen, wit Verhand. Batavia. T.W.IX deel, 1822.

Comment 10 p. 82 in SKR cites an article by Sakaguchi 1970 on Youan's special interest in the method of the boiling point of liquids at different heights. In *Seimi Kaiso* Youan presents exemples of five foreign mountains, boiling temperature on those mountains and their heights given in English and in Japanese units.[87]

19. ○ Niewenhuis, See no. 7.

20. ○ Dutch Pharmacopeaia 1826.

『和蘭局方』 *Waran (Oranda) Kyokuhou*. Dutch Pharamcopeia.

Nederlandsche apotheek 's-Gravenhage (The Hague) 1826.

This work is cited 15 times in *Seimi Kaiso*, about manufacturing and properties of substances that are mainly used in pharmacy. e. g., property of potassium carbonate,[88] and its manufacturing.[89]

21. ○ Richerand, A., New basics of the physics of human physiology 1826.

『利氏身窮理篇』 *Rishi Sinkyurihen*. Mr. Richerand's study of the physical laws of the human body.

Nieuwe grondbeginselen der Natuurkunde van den mensch. Amsterdam, 1826.

French origin: *Nouveaux elements de physiologie*, Paris 1801, Dutch translation by A. Van Erpecum, 1821 and 1826.[90]

In chapter 51 on "water containing vapor, vapor containing water", Youan comments saying that "according to Richerand, in water, there is a kind of gas, inner water is used in animal breathing, and the sense of hearing is affected. If you put fish in a bell exhausted of air, the fish dies. Also, insert into glass bottle, hermetically seal its mouth, the same thing happens".[91] Youan also cites Richerand's book on Human Physiology in a chapter on bismuth and other metals.[92]

22. ○ Catz Smallenburg, F. van, Chemistry Study Book.

『蘇氏舎密』 *Su shi seimi*, Mr. Su's chemistry.

Leerboek der scheikunde. Leiden, 1827-1829.

There are forty eight citations of Catz Smallenburg (1781-1848) in *Seimi Kaiso*. Youan could have acquired there the most advanced chemical ideas such as Berzelius' electro-dualism. Catz Smallenburg cited many authors, including Davy, Bergman, Gmelin, Döbereriner, Meinecke and others (1833 Leyden edition). Mac Lean mentions Catz Smallenburg Chemistry book presence in Deshima in 1837, it was brought on the ship *De Twee Cornelissen*.[93] The book was found in Udagawa House old possessions. Humphry Davy (1778-1829) is cited 15 times. Davy is cited concerning his use of the powerful Volta column and the isolation of Kalium. (See Rees no. 24 below).[94] Azuma found in Kyō-U library unpublished manuscripts by Youan. Azuma suggested that Youan was exploring the possibility of publishing a chemical book titled *Kaibutsu Engen-ko* 開物淵原稿, based on the content of Smallenburg's chemical book.[95] According to Tsukahara, Youan cites the work mainly from its practical and experimental parts; not advanced scientific theories but a more reflection of popular issues by a pragmatic chemist whose theoretical discussions were rather superficial.[96]

Interestingly, in the next section on Kalium, "that is also called potassium", Youan cites together the books by Ypey 『広義』, Smallenburg 『蘇氏舎密』 and Niewenhuis 『紐氏韻府』.[97] One may imagine Youan sits and those three books are opened in front of him, perhaps more than those three only. The text reflects Youan's professional approach to his study.

23. ○ Water, J. A. van de, Mr. Water's Pharmacy 1829

『窓多児氏薬論』 *Watarushi Yakuron*. Mr. Wataru's Pharmacy 1829.

Beknopt doch zoo veel mogelijk volledig handboek voor de leer der geneesmiddelen(materiamedica). Amsterdam, 1829.

Topics cited in *Seimi Kaiso* from Water's book include: Phosphoric acid,[98] magnesium carbonate,[99] potash and ammonia,[100] barium hydrochlorate,[101] and iodine.[102]

24. ○ Rees, W. van, A Report on Galvani. 1803.

『ガルヴァニ 紀事』 *Garubani Kiji* Galvani Account.

Verzameling van stukken, als bijdragen tot het Galvanismus, zoo in opzicht tot dezelfs genee- als natuurkundige werkingen, 2 dln (1st en 1803, 2nd en 1805), Arnhem, Moelman. [103]

End of Udagawa Youan's list.

Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Historical Articles

Capillary Electrophoresis and its Basic Principles in Historical Retrospect

Part 1. The early decades of the "Long Nineteenth Century": The Voltaic pile, and the discovery of electrolysis, electrophoresis and electroosmosis

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Abstract. Here we set forth the first from a series of reports devoted to the history of capillary electrophoresis. In this opening part, we go more than two centuries back in time and revisit original discoveries of electrolysis, electrophoresis and electroosmosis. We emphasize the essential role of a brilliant invention of 1799 by Alessandro Volta, the Voltaic pile, basically the first battery delivering a constant-flow electricity, which has made all the scientific advances in the subsequent years and decades possible. We describe the experiments of William Nicholson and Anthony Carlisle revealing electrolytic decomposition of river water followed by enlightened investigations by Nicolas Gautherot, Ferdinand Frédéric Reuss and Robert Porrett that each independently and unaware of the works of the other uncovered the phenomena of electrophoresis and electroosmosis. We give not only a technical description and a chronological overview of the inventive experiments, but offer also some formidable details as well as circumstances surrounding some of the initial inventors and their observations. We conclude this time period, for which we coin the term "*1st epoch of electrophoresis*", with the same year 1914 as the astonishingly coincident period of the European history between the French revolution in 1789 and the begin of the First World War, termed the "*Long 19th Century*" by the British historian Eric Hobsbawm. We accentuate the surprising fact that over this entire cycle of 125 years no attempts were taken to utilize the findings and newly acquired knowledge to perform an electric driven separation of compounds from a mixture. In the field of electrophoresis and electroosmosis, it is rather the epoch of pure than of applied science.

Keywords: capillary electrophoresis, history, discovery, electroosmosis, electrolysis.

OPEN ACCESS

Citation: Kenndler E., Minárik M. (2021) Capillary Electrophoresis and its Basic Principles in Historical Retrospect - Part 1. The early decades of the "Long Nineteenth Century": The Voltaic pile, and the discovery of electrolysis, electrophoresis and electroosmosis. *Substantia* 5(1) : 119-133. doi: 10.36253/Substantia-1018

Received: Jul 10, 2020

Revised: Aug 30, 2020

Just Accepted Online: Aug 31, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

PREFACE

Electrophoresis is the motion of electrically charged particles, which are dispersed in a liquid, and which drift relative to the fluid under the influ-

ence of a spatially uniform electric field.[1] *Capillary Electrophoresis* is the version of electrophoresis in which the liquid is inserted into a narrow open tube.[2] The liquid dispersion can be a solution of ions, an emulsion or a sol of colloids or – in rarer cases – a suspension of coarse granular particles, a noteworthy differentiation, which is often ignored (colloids do not form solutions; please pay heed to footnote[3]). Notwithstanding that nowadays electrophoresis is nearly exclusively used as a separation method, the term *electrophoresis* classifies the electrically driven movement of charged particles of any size in liquids *in a general meaning*.

Electroosmosis (also named electro-endosmosis in the past), can be seen as the reverse phenomenon compared to electrophoresis. Hence, electroosmosis is the motion of a liquid around an electrically charged surface in response to an applied electric field.[4] The electrically charged surface could be an inner wall of a capillary, a membrane, a porous plug or an immobilized set of particles. Electroosmosis plays an important role in practical capillary electrophoresis, because its flow velocity adds to the velocity of the charged particles.

Review papers on capillary electrophoretic methods (e.g. refs. [4-8] and others) usually mention only briefly the historical background on which the electrically induced migration of ions or colloidal particles is based. Although we assume that in the majority of cases in practice the basic principles are known, we wish to lay out an investigatory tale of the development of the technique from a more general perspective including some noteworthy historic facts such as that – contrary to the widely accepted perception – electrophoresis in open narrow glass tubes with a few hundred micrometers inner diameter (today widely recognized as capillary electrophoresis) is not an invention from the 1960s. In fact, it was first systematically carried out nearly a century prior, *viz.* in the years 1860 and 1861 by T. Jürgensen[9] and by G. Quincke,[10] albeit not for separation purposes. Thus, we here put forward a retrospect of the history of (capillary) electrophoresis from a broader perspective.

In our opinion, this long period can be subdivided into three distinctive epochs:

In the *1st epoch* of electrophoresis (whereby *electrophoresis* was not named as such over the entire period[5]) the focus of the research was directed to the basic physical and chemical principles, hypothesis, theories and laws of the electrically-induced migration of charged particles in liquids, and of the electroosmotic movement of liquids. The decisive characteristic of this *1st epoch* is a lack of intent to use electrophoresis as a separation method. This epoch commenced by the discoveries of electrolysis, electrophoresis and electroosmosis. It was rendered possible by an invention of a tool that enabled these discoveries, *viz.* a source of constant-flow electric-

[1] We do not always use the IUPAC recommendations (ref. [1] IUPAC, *Compendium of Chemical Terminology Gold Book*, Online version https://goldbook.iupac.org/ ed., **2014** [2] T. A. Maryutina, E. Y. Savonina, P. S. Fedotov, R. M. Smith, H. Siren, D. B. Hibbert, in *IUPAC Recommendations*, Pure Appl. Chem. ; 90(1): 181–231, **2018**.) because in some (rare) cases they are incomplete, ambiguous or out-of-date.)

[2] It is a matter of fact that no general definition exists for the inner diameter (i.d.) a narrow tube must possess to be considered as a capillary. In separation methods capillaries are open tubes with i.ds. of about 100 to 300 µm in capillary gas chromatography (GC), at the time of the replacement of the packed GC columns (which had i.ds. of 2 to 5 mm) by capillaries, so-called wide bore capillary columns with i.ds. of 540 µm were commercially offered. In modern instrumentation of capillary electrophoresis, the open tubes have i.ds. ranging from 25 to 100 µm, but also those with 5 µm i.d. are applied in zone electrophoresis and of 300 µm in isotachophoresis. The dimensions mentioned serve only as orientation for the reader.

[3] We use the following terminology for these liquid systems in the present paper:
The generic term for the different types of the particle-solvent systems is *dispersion*. In a *dispersion* the particles are distributed in a *continuous medium* (in electrophoresis the *continuous* medium is usually a liquid). Depending on the size of the particles, the following kinds of dispersions can be differentiated: (i) *solutions*, (ii) *emulsions* and *sols,* and (iii) *suspensions*.
ad (i) Small particles of molecular size (with typical radii in the range of several 10^{-10} m) *e.g.* stemming from electrolytes (either from solids like salts or from liquids like some pure low molecular weight saturated carboxylic acids) form a *homogeneous* mixture with the liquid, which is termed *solution*.
ad (ii) A *colloid*, synonymously termed *colloidal system* or *colloidal dispersion*, consists of a *heterogeneous* mixture of two phases, where the dispersed particles - *which are also named colloids* - are larger than small ions; their sizes range between about 10^{-9} and 10^{-6} m. Solid colloidal particles dispersed in liquids form *sols*, liquid colloidal particles form *emulsions*. Note that a colloidal system consists – in contrast to solutions - of two different phases, which are separated by an interface.
ad (iii) Particles which are larger than colloids form *suspensions as heterogeneous dispersions* in the liquid. Other than solutions and colloids, the particles sediment during long-standing periods. A special case are *gels* in which liquids are dispersed in solids.

[4] Other electrokinetic phenomena are the streaming potential and the streaming current, the sedimentation or centrifugation potential gradient, the colloid vibration potential, and the electrokinetic sonic amplitude. They do not play a role in the present topic. Readers are referred for details to ref. [3] J. Lyklema, *Fundamentals of Interface and Colloid Science. Vol. II: Solid-Liquid Interfaces, Vol. 2*, Academic Press, London, San Diego, **1995**.

[5] As typical examples we mention that F. Kohlrausch, who was one of the leading scientist in the area of ion migration, entitled his paper from 1893 "Über die Geschwindigkeit elektrolytischer Ionen" (*On the velocity of electrolytic ions*), ref. [11] F. Kohlrausch, *Ann. Phys. Chem.* **1893**, *50*, 385-408., and that from 1897 which served as base for the understanding of the different electrophoretic separation methods "*Ueber Concentrations-Verschiebungen durch Electrolyse im Inneren von Lösungen und Lösungsgemischen*" (*On concentration shifts due to electrolysis inside solutions and mixtures of solutions*"), ref. [12] F. Kohlrausch, *Ann. Phys. Chem.* **1897**, *Neue Folge Band 62*, 209-239. Both papers deal with ion migration, not with electrolytic processes.

ity by Alessandro Volta in 1799, the Voltaic pile, which transformed chemical into electric energy. However, we let this *1st epoch* begin even earlier, that is to say by the initiation of Volta´s ideas of a new kind of electricity which contrasted the misinterpretation of the well-known frog experiments by Luigi Galvani in the 1780s.

During the 125 years following Galvani´s experiments and the invention of Volta´s pile electrophoresis was applied solely to study the physical and chemical properties of pure compounds. Surprisingly, although all principles that govern the migration of ions and of dispersed colloidal particles in free solutions[6] were already progressively known, no attempts were made to apply them to separate constituents of mixtures. This *1st epoch* lasted until the midst of 1910 with the first intentional use of electrophoresis to perform separations in free solutions and it is the time period covered by the first series of our historical expeditions.

Due to his reading of the political works of the prominent British historian Eric J. E. Hobsbawm,[7] one of the present authors (E.K.) ascertained the remarkable coincidence of the duration of this *1st epoch* with an era referred to as the "*Long 19th Century*" in political sciences, specifically the time between the French revolution in 1789 and the begin of the First World War in 1914. The term *Long 19th Century* was introduced as a kind of a clamp for the first three volumes of Hobsbawn's tetralogy on the history of the 19th and of the 20th century.[13-15] Due to this astonishing temporal co-occurrence we have adopted the term *Long 19th Century* for the *1st epoch* of electrophoresis, which is the topic of the first series of our retrospect.

The *2nd epoch* of the history of (capillary) electrophoresis we let begin with its first intended utilization as separation method in the midst of 1910, and this period lasted till the 1990s. For the *2nd epoch* of electrophoresis we adopt the name, the "*Short 20th Century*", from the fourth volume of Eric Hobsbawn's tetralogy entitled *The Age of Extremes: The Short Twentieth Century, 1914–1991*.[16] In this book, the *Short 20th Century* was defined as the time period between the begin of the First World War in 1914 and the collapse of the USSR in 1991. During this time various electrophoretic methods were developed for the separation of ionic and colloidal particles. A notable highlight was the spectacular separation of serum globulins by Arne Tiselius in 1937 (awarded the Nobel Prize in 1948) by using the moving boundary method in free solution (which is one of the variants of electrophoresis). Nota bene that the separation followed the principles of the "*beharrliche Funktion*", the *regulating function*, derived by Friedrich Kohlrausch already in the previous *1st epoch* (*viz.* in 1897).[12]

It is to mention that during the first part of this *2nd epoch* most of the electrophoretic separations were not carried out in free solutions, they applied supporting or separating materials like paper, gels, etc. In contrast, our main interest is directed on electrophoresis in the capillary format in free solution, which was introduced in the 1960s. This method obeys the laws of the traditional electrophoresis in free solution, those which were derived during the *Long 19th Century*, and were refined at the begin of the *Short 20th Century* (e.g. by the concept of the chemical activity).

In the 1960s several variants of capillary electrophoretic techniques were established, mainly by the pioneering work of Frans Everaerts and his coworkers in Eindhoven with isotachophoresis (see e.g. ref. [17]), and by Stellan Hjertén in Uppsala with zone electrophoresis (see e.g. ref. [18]). These methods, (interestingly both were suggested by Nobel laureates, A. J. P. Martin and Arne Tiselius, respectively) were performed in open narrow-bore tubes with inner diameters down to ca. 200 µm. At this time, isotachophoresis (persistently called *displacement electrophoresis* by Hjertén), became the dominant variant, while capillary electrophoresis itself played only a niche role compared to well-established chromatography and gel-based electrophoresis.

[6] We are fully aware of the fact that only ions can be dissolved and exist then in free solution, but colloidal particles are forming emulsions or sols, not solutions. However, we further use for the sake of convenience the attribution *free solution* also to colloidal particles.

[7] Eric John Ernest Hobsbawm [1917 (Alexandria, Egypt) - 2012 (London)] was a British historian with marxist orientation. The family of his father, named Obstbaum (verbatim in English translation "fruit tree"), had migrated from Austria to Great Britain and modified the name to Hobsbawm. The father got a position in the Sultanate Egypt, which was a British protectorate at that time. His mother came from a wealthy Viennese family. After the First World War, the family went back to Vienna, where they lost their assets due to the gigantic inflation at that time. After the death of his parents (the father died in 1929, the mother in 1931) relatives took Hobsbawm to Berlin, where he came in contact with the German Communist Party. In 1933, the family went to London. For the following years Hobsbawm received a stipend for Cambridge, where he became member of Communist Party of Great Britain, what he remained livelong. In 1947, Hobsbawm became lecturer at an evening school at London University, the Birkbeck College. During this time, he published Jazz-critiques in *New Statesman* under the pseudonym Francis Newton. After publication of his tetralogy between 1962 and 1987 (ref. [13] E. J. E. Hobsbawm, *The Age of Revolution: Europe: 1789–1848*. Ref. [14] E. J. E. Hobsbawm, *The Age of Capital: 1848–1875*. Ref. [15] E. J. E. Hobsbawm, *The Age of Empire: 1875–1914*. Ref. [16] E. J. E. Hobsbawm, *The Age of Extremes: The Short Twentieth Century, 1914–1991*) about the history of the 19th and the 20th century he became known worldwide. For the time period between 1789 and 1914, described in the first three volumes of his tetralogy, the term the *Long 19th Century* was coined, that described in the fourth volume over the years between 1914 and 1991 was termed the *Short 20th Century*. Not until 1971 he was appointed professor in London, where he died at the age of 95.

The *2nd epoch* was concluded in the late 1980s with the advent of a new capillary material – amorphous quartz, named fused silica – which has led to a sudden increase of interest in the separation methods community. The favorable mechanical, optical and surface properties of this material, which has extensively been exploited in gas chromatography, facilitating an enormous separation capability and a highly sensitive detection of extremely low quantities of analytes, prompted the commercial availability of a number of different user-friendly instruments. Consequently, capillary electrophoresis, especially the *zone electrophoresis* version, became a member of a family of the high performance separation methods

The *Short 20th Century*, lasting only 75 years from midst 1910s to about 1990, was followed by that we are consequently terming the *3rd epoch* of electrophoresis, which brought an outstandingly large number of innovative experimental and instrumental approaches as well as novel applications. Coupling to mass spectrometry has brought a new dynamic to capillary zone electrophoresis. Perhaps the outmost notable achievement was a transfer of the classic size-based separation of DNA fragments from slab-gel electrophoresis into capillary electrophoresis mainly enabled by the introduction of linear entangled polymers as replaceable sieving matrices. This progress enabled, to mention only one well-known example, the execution of the Human Genome Project, which started 1990 and completed officially 2003 with the determination of the entire DNA sequence of the euchromatic human genome.

In the present *3rd epoch*, capillary electrophoresis is an indispensable tool in nearly all scientific disciplines, in life sciences for instance in genomics, proteomics and metabolomics. Since current research is the topic of this *3rd epoch*, we will not include it in our historical retrospect. This time is rather the theme of a topical, not of a historical review.

Following this brief overview, we will now return back to the dawn of the discoveries of the electric phenomena in the early decades of the *Long 19th Century*, to the period between the late 1780s and the midst 1810s.

AT THE TURN TO THE *LONG 19TH CENTURY*

Until the end of the 18th century the sources of electricity were electrostatic generators or electrostatic machines. These devices transformed mechanical work into electrical energy by a process of generation of charge by friction and induction. One such a device was invented in the 1760s by the Swedish physicist Johan Carl Wilcke (Wilke in his papers written in German language)[19-21] and re-invented and improved by Alessandro Volta in 1775 who named it *elettroforo perpetuo*. It was a simple generator of static electricity by induction, which became very popular as *electrophore* or *electrophorus*.[8]

A drawing of an *electrophore* is shown in Figure 1. It consists of two plates.[9] The bottom plate, the *cake* or *sole*, is a dielectric, *i.e.* an electrically non-conductive material. A detailed instruction for the preparation of an electrophorus in a book from 1814[23] recommends a resinous "*cake*" of about half an inch thickness, formed by melting equal parts of resin, shellac and Venice turpentine[10] together. The upper part (the "*cover*") is a metal plate with an insulated handle, comparable with the plate of a capacitor. Electricity is generated by electrostatic induction (see footnote 11).

The generated electricity could be stored e.g. at a special cylindrical capacitor, the *Leyden jar*. Though this

[8] We have chosen this device, because it demonstrates the principle of the generation of static energy in a very simple form. In addition, we accentuate that the term *electrophore* points to the little known fact that a word combined from Greek ήλεκτρον (*'elektron'*), and φέρω (*'phero'*), freely translated as "*the bearer of electricity*", was in use already in the 18th century. It was not a new term when it was introduced at the begin of the *2nd epoch* of electrophoresis for the method under discussion in the present paper.

[9] An early description of this popular device is given e.g. in Chapter IV, p. 380.389, from ref. [22] T. Cavallo, *A Complete Treatise of Electricity in Theory and Practice with Original Experiments*, Edward and Charles Dilly, London, **1777** or later in 1814 on p. 121-122 of ref. [23] G. J. Singer, *Elements of Electricity and Electro-chemistry*, London, **1814**. In this book, a large number of practical experiments are describes. A further description is given in the section "*L´Électrophore*" in *Chapitre IX. Des Electricités dissimulées*. in Vol. 1, pp. 571-575, of Jean-Baptiste Biot´s textbook of experimental physics from 1821 (1st ed. in 1817, (ref. [24] J.-B. Biot, *Précis élémentaire de physique expérimentale. Tome I, Vol. 1*, 2nd ed., Deterville Paris, **1821**.)

[10] Venice turpentine is a highly viscous oleoresin, a mixture of bicyclic diterpenoid compounds, mainly with carboxylic and alcoholic functional groups. It is collected from the exudate of the European larch in Tyrol, Austria. It must not be confused with oil of turpentine, which is a mixture of liquid monoterpenes.

[11] For the generation of electricity, the upper surface of the earthed bottom resin plate becomes negatively charged by rubbing, e.g. by a piece of dry fur (cat´s skin is the best, according to ref. [23]), or a piece of wool. Then, the metal plate is placed on the "*cake*", and becomes positively charged by induction at the surface directed towards the cake, and negatively at the opposite surface of the metal. The plate is taken off from the cake, then the upper, opposite surface is touched with a finger, causing the transfer of the negative charge to ground. At the metal plate only the positive charge formed by induction remains. It can then be, for example, transferred to a Leyden jar. This operation can be repeated many times without the need to rub the resin again, and was therefore termed by Volta *elettroforo perpetuo* (*perpetual electrophorus*).

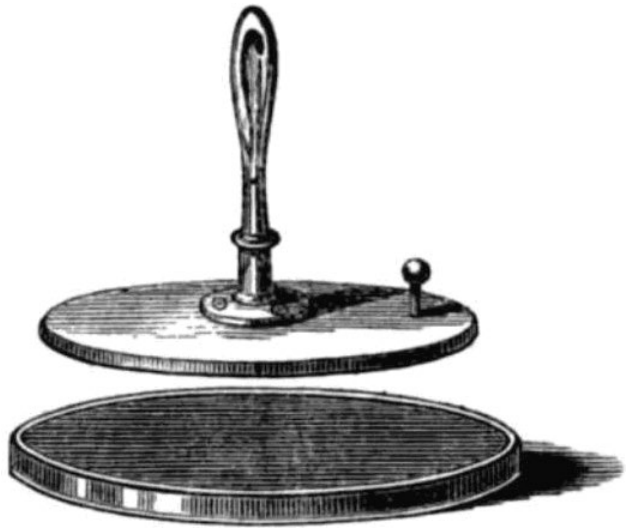

Figure 1. Drawing of an *electrophore* or *electrophorus* (Volta termed it *elettroforo perpetuo),* a device for the generation of static electricity by induction. For explanation, see footnote 11. Taken from ref. [25].

capacitor was able to deliver large electric potentials,[12,13] it's capacity to store static electric energy was low, which required frequent recharging for use over longer period of time. The capacity could be increased by connecting several jars in parallel, forming a *Leyden battery* in this way.[14] However, the need for recharging remained and, further to its disadvantage, the discharge current did not remain constant.

A new aspect for the generation of electricity was unintentionally opened up by Luigi Galvani´s false conclusions of his experimental results. Galvani,[15] professor of anatomy in Bologna, investigated since early 1780s the effect of electricity on animals. Galvani´s findings, which he misinterpreted, were the prelude for the invention of a revolutionary new source of electricity by Alessandro Volta. The story began with an observation of one of Galvani´s students, who touched with the tip of a scalpel a lumbar nerve of a dead skinned frog which was placed nearby an electrostatic machine. This accidental contact caused a convulsive twitching of the frog´s legs as if alive. Galvani, who assumed a context of this contraction with electricity, commenced in 1789 a series of experiments by which he noticed that the muscles of the frog´s legs were contracted even in the absence of an electrostatic machine. They also twitched when they were connected by contacts made of two different metals (e.g. of copper and iron).[16] Galvani postulated that the source of this contraction was a new kind of electricity, which he termed *animal electricity*. He believed that this new energy was intrinsic to the body of the dead frog, and hypothesized that the frog´s brain produced electricity, and its body acted as a kind of electric condenser. He further assumed that the nerves are the conductors which transmit the electricity to the muscles. Galvani published his findings in 1791 in "*De viribus electricitatis in motu musculari commentaries*" ("*Commentary on the Effects of Electricity on Muscular Motion*"),[28] which attracted extraordinary attention by his scientific colleagues, amongst them also by Alessandro Volta.[17]

THE VOLTAIC PILE

Volta was highly experienced in the field of static electricity, and was initially convinced by the existence of the animal electricity, but since summer 1892 he began to doubt Galvani´s hypothesis of animal electricity. Volta, in contrast, supposed, that the source was the contact electricity originating from the metallic wires in connection with the interposed body fluid of the frog as a conducting medium. He executed experiments with different metals and was able to measure even very low quantities of electricity when the metals were brought into mutual contact.[18] [31]

[12] In the literature, *voltage* has normally been used to describe the *electric potential difference*. According to IUPAC "*…this term is discouraged, and the term applied potential or (electric) potential should be used instead for non-periodic signals…*" (PAC, 1985, *57*, 1491 *(Recommended terms, symbols, and definitions for electroanalytical chemistry (Recommendations 1985)),* p. 1505). In order to avoid confusion, we prefer to use the term (*electric*) *potential difference* if appropriate.

[13] With replica of historical Leyden jars potential differences of several ten thousand Volt were obtained.

[14] We consider *n* capacitors, *i*, connected in parallel, and use the symbols *U* for potential, *Q* for charge, and *C* for capacity. Then $U_{tot} = U_i$; $Q_{tot} = \Sigma Q_i$; $C_{tot} = \Sigma C_i$. This connection in parallel is applied to increase the capacity of the Leyden battery. Upon discharging of the capacitor, the discharge current, *I*, decreases exponentially with time, *t*, according to $I(t)=-I_0 e^{-t/t}$; *t* is the time constant of the discharge process.

[15] Luigi Aloisio Galvani [(1737) Bologna, Papal States, at present Italy - 1798], (in Latin *Aloysius Galvanus*) was an anatomist, physician, physicist, physiologist and biologist.

[16] Very detailed descriptions of Galvani´s observations are given e.g. in ref. [26] E. Du Bois-Reymond, *Untersuchungen über thierische Elektricität, Vol. 1*, G. Reimer, Berlin **1848**., and in ref. [27] O. E. J. Seyffer, *Geschichtliche Darstellung des Galvanismus*, J.G. Cotta, Stuttgart und Tübingen, **1848**.

[17] Alessandro Giuseppe Antonio Anastasio Volta, [1745 (Como, at present northern Italy) - 1827], since 1810 Count Volta; since 1774 professor of physics at the Royal School in Como, and professor in natural philosophy, and chair in experimental physics at the University of Pavia since 1819.

[18] The measurement of very low quantities of electricity was possible by a device which combined a condenser – which Volta constructed and built – with an electrometer created by Tiberius Cavallo (described in his book, ref. [22] T. Cavallo, *A Complete Treatise of Electricity in Theory and Practice with Original Experiments*, Edward and Charles Dilly, Lon-

He also found that the quantity of the generated electricity was higher when the two metals were separated by a third, non-metallic conductor, for example by a 2nd class conductor like a piece of paper soaked with salt solution. [32, 33] Thus, Volta argued that the nerves in Galvani´s experiments were stimulated by the electricity delivered by the communicating metals,[34] not by animal tissues, and believed in what he coined *metallic electricity* instead of Galvani´s *animal electricity*.

The debate between the two scientists ultimately led to the refusal of Galvani´s idea of an animal electricity (which was, with Volta´s generous agreement, further named *galvanic electricity*, and its topic *galvanism*; for details, see e.g. ref. [35]). The seminal result of Volta´s investigations of his metallic electricity was the creation of an electric element, which transformed chemical into electric energy.[19]

In contrast to the Leyden jar, Volta´s device enabled the generation of a continuous and constant-flow electricity. In 1800 Volta described the battery, a stack of assembled electrochemical elements later named *Voltaic pile*, in a detailed paper titled "*On the Electricity excited by the mere Contact of conducting Substances of different Kinds*".[37] He sent the description of the pile as a letter dated March 20, 1800 to the President of the Royal Society, Sir Joseph Banks. The letter was read June 26, 1800 befor the Royal Society in London.[38] Following we include the verbatim reproduction of the first two paragraphs from Volta´s letter to Banks with its exemplary clear description of the battery, and illustrate this explanation in Figure 2.

> …In prosecuting his experiments on the electricity produced by the mere contact of different metals, or of other conducting bodies, the learned Professor was gradually led to the construction of an apparatus, which in its effects seems to bear a great resemblance to the Leyden phial, or rather to an electric battery weakly charged; but has moreover the singular property of acting without intermission, or rather of re-charging itself continually and spontaneously without any sensible diminution or perceptible intervals in its operations. The object of the present paper is to describe this apparatus, with the variety of constructions it admits of, and to relate the principal effects it is capable of producing on our senses.
> It consists of a long series of an alternate succession of three conducting substances, either copper, tin and water; or, what is much preferable, silver, zinc, and a solution of any neutral or alkaline salt. The mode of combining these substances consists in placing horizontally, first, a plate or disk of silver (half-a-crown, for instance,) next a plate of zinc of the same dimensions; and, lastly, a similar piece of a spongy matter, such as pasteboard or leather, fully impregnated with the saline solution. This set of threefold layers is to be repeated thirty or forty times, forming thus what the author calls his columnar machine. It is to be observed, that the metals must always be in the same order, that is, if the silver is the lowermost in the first pair of metallic plates, it is to be so in all the successive ones, but that the effects will be the same if this order be inverted in all the pairs. As the fluid, either water or the saline solution, and not the spongy layer impregnated with it, is the substance that contributes to the effect, it follows that as soon as these layers are dry, no effect will be produced."

As depicted in Figure 2 any number of elements can be combined in order to increase the total electric potential of the pile. In his letter Volta described that each element consists of a pair of discs made from three materials, *viz*. from two different metals and a layer of a matter wetted with water or saline solution; the elements can be stapled about each other.[20] At the uppermost and the lowermost disc, respectively, metal wires are attached, and each of these elements contributes additively to the electric potential of the pile by its individual potential which depends on the kind of the metals.[21] Effects of

don, **1777**. Volta´s device was presented for the Royal Society in London, read March 14, 1782, entitled "*Del modo di render sensibilissima la piu debole Elettricità sia Naturale, sia Artificiale*" (ref. [29] A. Volta, *Phil. Trans. Roy. Soc. (London)* **1782**, *72*, 237-280. ("*Of the Method of rendering very sensible the weakest Natural or Artificial Electricity*") and ref. [30] A. Volta, *Phil. Trans. Roy. Soc. London. Part I* **1782**, *72*, 453 (vii-xxviii). This sensitive device was also essential for Volta´s research on his pile.

[19] Sir Humphry Davy, [1778 (Penzance, Cornwall, England) - 1829], teacher and mentor of Michael Faraday, said Volta's work was "*an alarm bell to experimenters all over Europe*" (see *e.g.* ref. [36] C. Russell, in *Chemistry World, Vol. 1 August 2003*, Royal Society of Chemistry, **2003**.).

[20] In each single Volta element in Figure 2 zinc is oxidized to Zn^{2+}, and releases 2 electrons. For the electrochemical reduction at the silver electrode several reactions are possible. If the silver plate is covered by a layer of silver oxide or silver salt (as it is when e.g. used half-a-crown coins are applied, as mentioned in Volta´s letter), Ag^{+} can be directly reduced. In absence of silver ions, e.g. when the plate is polished, oxygen from air or hydrogen ions from the impregnation solution can be reduced.

[21] In the Voltaic pile the elements (also named cells) are connected in series, i.e. the plus pole of the one element is connected with the minus pole of the adjacent element. All elements are flown through by the same current, which has the disadvantage that it is determined by the element with the lowest current. In the worst case the potential fails if one element is defective. The total electric potential difference of the series of elements of the battery is equal to the sum of the potentials differences of its single elements. The potential difference of an element made for instance from zinc and copper is about 1.1 Volt. Thus, in a staple of say 10 elements the applied potential is about 11 Volt between the two extreme discs.
When the elements are connected in parallel (which is not the case in the Voltaic pile), i.e. when the plus pole of the one element is connected with the plus pole of the adjacent one, and the minus pole with the minus pole, the load capacity of the battery (in A.h, Ampere hours) is the sum of the load capacities of the single elements. The total electric

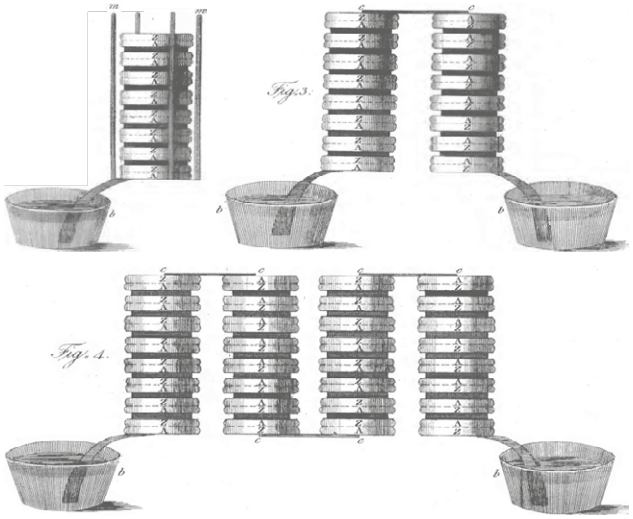

Figure 2. Drawing of Volta´s piles combined from 8, 16 and 32 elements, respectively, consisting of pairs of zinc (Z) and silver (A) discs. In these piles, the elements or cells are formed by a pair made from two discs of different metals which are in direct contact, in this Figure of silver, A, and zinc, Z, communicating with the next pair by an interposed spongy matter (e.g. a piece of cloth, leather, or pasteboard) moistened with a salt solution. The elements are stapled one upon the other, here with a silver disc as the lowermost, and a zinc disc as the uppermost one. Taken from ref. [37].

electricity on solutions could be investigated by immersing the wires` tips of the pile into the liquid, where they act as the electric poles.[22]

At last, we want to contrast Volta's story of his triumphant scientific successes – among many other honors he was made a Count in 1810 by Napoleon after the conquest of Italy – with a largely unknown and rather tragic-comic story, which is a matter of the metallic electricity Volta discovered. As early as in May 1793 John Ribison, [1739-1805], a British professor of natural philosophy at the Edinburgh University (he was physicist and mathematician) reported a peculiar experiment in a letter[39] sent to Alexander Fowler, the editor of "*Experiments and Observations relative to the Influence Lately Discovered by M. Galvani, and commonly called Animal Electricity*",[40] (translated into German in ref. [41]). The experiment was carried out by Ribison`s son, who brought a piece of silver and a piece of zinc in contact with his tongue and felt a strong stimulus, similar to a taste. Ribison repeated this experiment and obtained the same result. He was aware about some curious discoveries which had been made in Italy some time ago, but he had no further knowledge of what was going on in the recent years.

We describe here only some of the experiments which he reported in the letter. In one particular experiment he felt the same irritation at the tongue as already mentioned above when he placed a piece of zinc, in contact with a piece of silver at any other part of the mouth, the nose, the ear, the urethra or the anus. Also he applied a piece of zinc onto a wound of a toe, and a piece of silver to the tongue, and each time when he brought the metals in contact he felt a painful irritation at the wound where zinc was placed. Next, Ribison applied a rod of zinc and one of silver to the roof of the mouth. Upon connecting the ends of the rods, he felt a painful, convulsive pruritus, together with a bright refulgence in the eyes. Finally, Robison made a number of pieces of zinc of the size of a shilling-coin and formed a roll with an equal number of silver shillings. He observed under certain conditions an intensified irritation at the tongue, which was increased when the tongue touched all pieces of the metals at the side of the roll, effects which sourced from contact electricity.

Ribison´s report ended with his regret that he was not able to continue his experiments due to his indisposition. One might speculate that under more favorable circumstances he possibly invented an electric battery even few years prior to Volta.

THE DECOMPOSITION OF WATER BY ELECTRICITY: THE DISCOVERY OF ELECTROLYSIS

Volta sent his above mentioned letter in two separate parts to Sir Joseph Banks in London. After receipt of the first part of this letter, Banks has shown its first pages to Antony Carlisle.[23] It was already known that electricity can be sensed as electric "shocks", e.g. when electrostatic batteries were getting in touch with wetted hands or with the tongue. Based on the description in the letter, Carlisle assembled a pile, and – together with his friend William Nicholson[24] – repeated on April, 30 several experiments which were described in Volta´s letter (a

potential difference of the battery is equal to the electric potential difference of the single elements in case of their connection in parallel.

[22] In the contemporary literature, the terms electrode and electrolyze were unknown; they were proposed about three decades later by M. Faraday. The term pole was used in analogy to the poles of a magnet.

[23] Anthony Carlisle [1768 (Stillington, England) – 1840], an English surgeon, was professor of anatomy of the Royal Society from 1808 to 1824. As a matter of curio we mention that Carlisle is probably the anonymous author of the gothic novel *The Horrors of Oakendale Abbey*, published in 1797 and previously attributed to "Mrs. Carver".

[24] William Nicholson [1753 (London) – 1815], an English chemist, founded the *Journal of Natural Philosophy, Chemistry and the Arts* in 1797 (known as *Nicholson's Journal*). It was the first English monthly scientific journal.

graphical interpretation of their experiments is shown in Figure 3). Nicholson subsequently reported[42]

> This pile gave us the shock as before described, and a very acute sensation wherever the skin was broken. Our first research was directed to ascertain that the shock we felt was really an electrical phenomenon. For this purpose the pile was placed upon Bennett's gold leaf electrometer, and a wire was then made to communicate from the top of the pile to the metallic stand or foot of the instrument. … In all these experiments it was observed, that the action of the instrument was freely transmitted through the usual conductors of electricity, but stopped by glass and other nonconductors. Very early in this course, the contacts being made sure by placing a drop of water upon the upper plate, Mr. Carlisle observed a disengagement of gas round the touching wire. This gas, though very minute in quantity, evidently seemed to me to have the smell afforded by hydrogen when the wire of communication was steel.

Being interested whether or not this release occurs also when the wires were placed separated from each other, in a series of experiments Carlisle and Nicholson filled river water into a glass tube, and plunged the two wires in a distance of several centimeters from each other into the water. Upon closing the electric circuit, an effect which surprised the experimenters was observed, *viz*. that at one of the wires a fine stream of bubbles of oxygen, at the other wire bubbles of hydrogen evolved.[25] After testing wires made of several different metals, the most distinct result was obtained with platinum or gold.

It was obvious that the evolved gases must originate from the water, but the question raised how the gaseous hydrogen or oxygen could be invisibly transported through the liquid water to the opposite pole when they were formed – as initially assumed – at one and the same pole and from the same individual water molecule (for more details of this anecdote the readers are recommended to ref. [36]).

Since it was evident that the two gases are products of the disintegration of water, Carlisle and Nicholson

Figure 3. An illustration of an experiment of electrolysis by William Nicholson and Anthony Carlisle on May 2, 1800, by decomposing water by electricity of a Voltaic pile. Taken from ref. [45], "*La Pile de Volta*", Chapter III, p. 629, Fig. 324.

observed for the first time an electrochemical decomposition, which was later – as proposed by Michael Faraday – termed *electrolysis*. Nicholson published the results[26] - prior to the publication of Volta´s letter[37] – in a paper entitled "*Account of the new Electrical or Galvanic Appa-*

[25] It has to be mentioned that the decomposition of water by electricity, albeit not by an electrochemical reaction, was already carried out prior to the invention of the Voltaic pile, viz. by electric machines. George Pearson reported in 1797 the experiments made by the Dutch chemists Adriaan Paets van Troostwyk and J. R. Diemann, assisted by John Cuthbertson (see ref. [43] G. Pearson, *Phil. Trans. Roy. Soc. (London)* **1797**, *LXXXVII*, 142-157.; and ref. [44] G. Pearson, *Journ. Nat. Philos Chem. & Arts* **1797**, *1*, 241-246.). Cuthbertson was a highly qualified maker of scientific instruments and Fellow of the Philosophical Society of Holland and Utrecht. In the cumbersome and laborious experiments electric sparks generated by a Leyden battery were induced in succession in liquid water, which was decomposed into gaseous oxygen and hydrogen in measures of one to two. After collecting a sufficiently large quantity of the liberated gases, a spark was sent through them, causing their inflammation and the reconversion into liquid water.

[26] The first public report about these experiments appeared in the "*Morning Chronicle*", a London newspaper, on May 30, 1800. The authors found this information in Otto Ernst Julius Seyffer´s "*Geschichtliche Darstellung des Galvanismus*", ("*Historical presentation of the galvanism*"), published in 1848, ref. [27] O. E. J. Seyffer, *Geschichtliche Darstellung des Galvanismus*, J.G. Cotta, Stuttgart und Tübingen, **1848**. The book contains about 640 pages and describes in detail the history of the galvanism, from its first observation by J.G. Sulzer as soon as in in 1760 in Berlin, and Galvani in 1790, till 1845, with addition of some sources till 1847. It describes the contributions of about 600 authors (including the source of their publications), it circumstantiates detailed experimental set-ups, procedures and results in many contributions, it describes the reception of the results, controversial discussions between the authors, and puts them into the historical context. This is, in the opinion of the authors, an enormous achievement of Seyffer, considering the difficulty to get access at that time to the large number of different German, English, French, Italian and Russian journals.

ratus of Sig. ALEX. VOLTA, and Experiments performed with the same" in 1801 (in the July 1800 issue) in *Journal of Natural Philosophy, Chemistry, and the Arts.*[42] It is to be stated that the observations which were made by Nicholson and Carlisle in April and May 1800 introduced electrochemistry as a new scientific discipline.

The spectacular invention of Volta (and the effect of electricity on the decomposition of water) was rapidly communicated by the scientist across Europe, and provoked an eminent impulse for research in this novel discipline. Although the fact of the decomposition of water at the poles was corroborated by the formation of the gas bubbles, the transport of the electricity[27] through the solution remained completely unintelligible.

In the course of the various experiments which were executed by numerous researchers in Europe other phenomena that could occur between the two poles of Volta´s pile were discovered. The observation of these phenomena was facilitated because they could directly be followed visually. It was the migration of dispersed coarse granular particles, and – under certain conditions – the electrically induced movement of the liquid. The former phenomenon is now known as *electrophoresis*, the latter as *electroosmosis*.[28]

THE DISCOVERY OF ELECTROPHORESIS AND ELECTROOSMOSIS BY N. GAUTHEROT, F.F. VON REUSS AND R. PORRETT

Until the midst of the 19th century, Robert Porrett[29] was accounted as the discoverer of electroosmosis. This attribution was based on a paper which he published in 1816, entitled "*Curious galvanic experiments*"[46] (in 1820 in German).[47] In one of the described experiments, Porrett divided a glass jar by a bladder, obtaining two separated chambers in this way. When he filled one chamber with water the other chamber remained dry even when left for several hours as the water did not penetrate the bladder. Next, Porrett put a few drops of water into the empty chamber, just covering its bottom. Then he used a Voltaic pile connecting the positive pole to the water-filled chamber and the negative pole to the chamber with wet. Porrett then observed that water was transferred from the water full chamber through the bladder divider into the nearly empty chamber, resulting, within half an hour after completion of the electric circuit, in equal water levels in both chambers. This transport process further continued, raising the level in the negative chamber ¾ of an inch above the level of the positive chamber. Without having an explanation for this phenomenon Porrett named it *electro-filtration*. With our hindsight it is evident that the water transport observed by Porrett was by electroosmosis due to the electric double layer formed in the pores of the bladder.

The discovery of electroosmosis has been attributed to Porrett until the midst of the 19th century, when Otto Ernst Julius Seyffer referred to two experiments by Ferdinand Frédéric Reuss (Ferdinand Friedrich von Reuß)[30] published about one decade prior to Porrett, but which up to that moment were nearly unnoticed so far by the majority of the scientific community. In his book from 1848 Seyffer identified Reuss as the discoverer of electrokinetic phenomena.[27]

As reminded by Seyffer, Reuss described the execution of two experiments in publications which appeared in a Russian journal in 1809 (in French)[48] and in 1821 (in Latin).[49] The first publication was entitled "*Sur un nouvel effet de l'électricité galvanique",* the paper from 1821 *"Electricitatis Voltanae potestatem hydragogam tanquam novam vim motricem, a se detectam, denuo proposuit ejusque in naturae operibus partes investigare tentavit*" (Reuss was well-known for his chemistry lectures at the University held in Latin). In the first experiment Reuss packed quartz sand between two platinum wires (*a* and *b* in Fig. 4, top drawing) positioned as electric poles at the bottom of a V-shaped quartz tube. The tube was filled with degassed water, and the platinum wires were connected to a Voltaic pile. After closing the electric circuit, Reuss observed the already known decomposition of water under formation of gaseous oxygen and hydrogen at the poles.

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[27] At that time and later the flow of the electric current was named *the transport of electricity*. However, transported are the charges, either by the electrons in 1st class conductors like metals, or by ions or other charged particles in 2nd class conductors, e.g. in electrolyte solutions or in melted salts. For historical reasons, we temporarily also use the term *transport of electricity.*

[28] It is pointed out that the terms *electrode*, *electrolysis*, *electrophoresis*, *electroosmosis*, to name a few, were not known at that time. The first use of the term electro-endosmosis or electroosmosis was initiated in the 1830s, the term electrophoresis one century later**.** We use these terms (ahistorical) in the present paper when it serves for its better readability.

[29] Robert Porrett Jr. [1783 (London) – 1868] was chief administrator of the armory of London Tower by profession. He was member of the Society of Antiquaries and Fellow of the Chemical Society. Interested in chemistry and physics, he obtained thiocyanic acid from Prussian blue (Berlin, Parisian, Paris or Turnbull's blue, Iron(II,III) hexacyanoferrate(II,III)) upon reaction with potassium sulfide. and examined, amongst others, the chemistry of compounds containing iron and cyanide.

[30] Ferdinand Friedrich von Reuß [1788-1852] was born in Tübingen, Germany, where he studied medicine. He finished his studies in Göttingen as Dr. med. et chir. and became college lecturer for general medical chemistry in 1801. He became known for his investigations of the horse lymph, which was probably the cause for the assignment to a professorship at the Imperial University Moscow in 1808. In addition, he was professor for chemistry and pharmacography at the Imperial medico-chirurgical Academy from 1817 to 1839.

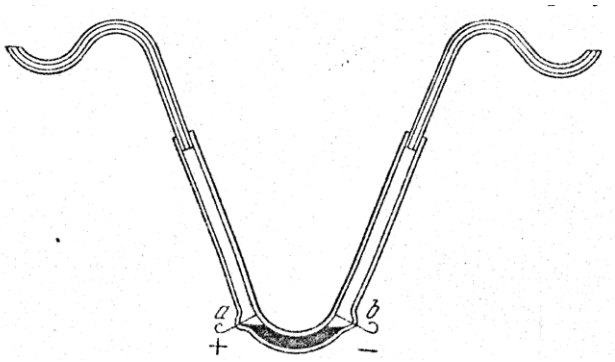

Figure 4. V-shaped quartz tube device used by Reuss in his first experiment on electroosmosis. The dimension of the tube was "*3 lignes de diamètre et de 7 pouces de lorgueur*", i.e. 3x2.26 mm in diameter and 7x2.706 cm in length. The stippled portion is quartz powder. *a* and *b* are the wires of the + and the – pole, respectively. Reproduced from Reuss, ref. [48].

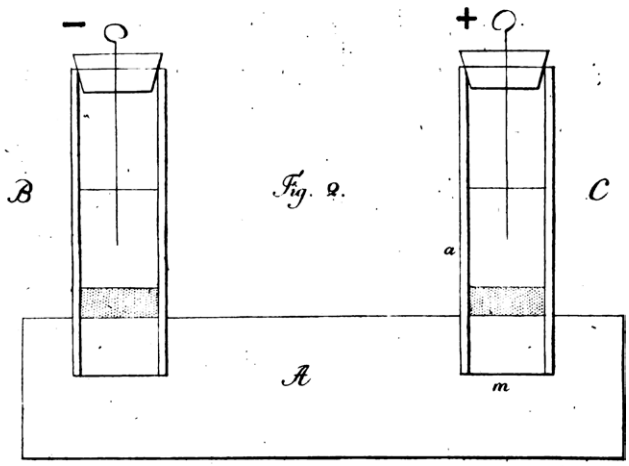

Figure 5. *B* and *C* are two water-filled tubes (of circ. 3 cm i. d., placed at a distance of about 14 cm), plunged in a clay base, *A*, the arrangement used by Reuss in his second experiment on electroosmosis. The stippled portions in *B* and *C* are sand layers. The wires of the + and the – poles are immersed into the water. Reproduced from Reuss, ref. [48]. For details, see text.

Even mor importantly, he also observed a slow rise of the water level at the side of the tube with the negative pole *b*, and an according decrease of the level at the positive pole *a*. Disconnection of the wires led to a reversal of the flow due to gravity, reconnection reproduced the initial effect of the rise of the water level. After fourteen hours, the tube at positive pole *a* was empty, while that at pole *b* was completely filled with water. After four days the experiment was concluded, the wires were disconnected and the initial state with the equal water levels was reconstituted within a few minutes. The results of this experiment unequivocally confirmed the occurrence of electroosmosis (here with a flow of the liquid towards the negative pole[31]), a phenomenon Reuss termed *motus stoechiagogus*.

In his second experiment Reuss filled a block of moist clay into a container (*A* in Figure 5) and inserted two glass tubes. The bottoms of the tubes were covered by thin layers of carefully washed sand (indicated by shaded zones in the Figure), and the tubes were filled by 3 cm with water. Platinum wires were inserted into the centers of the tubes through their open ends (plugged with corks), and were connected to a Voltaic pile.

After completion of the current, the clay first began swelling and softened to mud at the bottom of the tube with the positive pole; in the following the penetration of the sand layer by clay particles was observed under formation of a pointed mound at position *a* in the Figure. Muddy liquid was separating from the mound and formed a mud layer at the surface of the sand. This process occurred only in the tube with the positive pole, but no effect was noticed in the other tube. Upon continuation of the experiment for four days, electroosmosis of water from the positive to the negative pole was observed, most probably caused by the same source as in the first experiment, *viz.* by the negatively charged quartz sand (see footnote 31).

The question now arises whether electrophoresis or electroosmosis was causing the transport of the clay particles through the sand layer into the tube with the positive pole. Reuss attributed the penetration of the clay to electroosmosis. Since commonly the clay particles (like quartz

[31] In this experiment of Reuss, the electroosmotic flow of the liquid towards the negative pole has its cause in the negatively charged surface of the quartz sand which he inserted into the tube. The sand consists of silica, polymerized silicic acid, which possesses residual silanol groups at its surface. This groups have weak acidic property and dissociate in the presence of water into negatively charged silanolate groups (≡Si-O$^-$). These groups are the sources of the fixed negative charges at the surface of the quartz sand, which is the one side of the so-called electrochemical double layer. In electroosmosis positive ions dissolved in the liquid phase compensate the negative charges, but due to their thermal energy they are not rigidly attached at the solid surface, and always at least a fraction of the cations is freely movable. If a tangential electric field is applied, the free cations are attracted towards the cathode due to electric forces dragging a layer of water into the same direction, which represents the first water layer flowing by electroosmosis. Due to viscosity forces, the flow impulse is transferred to each adjacent water layer into the bulk of the liquid, and within shortest time the entire liquid is flowing by electroosmosis. In the present case of quartz, water is driven by the cations of the interfacial double layer towards the negative pole. It is interesting that electroosmosis was originally discovered by crystallized silica as described above, because the same material, in its amorphous form, named fused silica, is the universal choice for the common narrow open tubes used in contemporary capillary electrophoresis. In this separation method, the electroosmotic flow is a fundamental component of the separation set-up utilizing the migration of the analytes.

sand) are negatively charged,[32] electroosmotic flow caused by the porous clay of block *A* in Figure 3, bottom, would be directed towards the negative pole. By reason that the clay particles migrated towards the positive pole electrophoresis, not electroosmosis, was probably the cause of their migration. Thus, the first observation of electrophoresis had to be attributed to Reuss, and not to Porrett.

But by midst 19th century only few scientists drew attention to Reuss´ work, amongst others Gustav Heinrich Wiedemann and Georg Hermann Quincke, reputed scientists in the field. In a paper from 1861 entitled "*Ueber die Fortführung materieller Theilchen durch strömende Electricität" ("On the transport of material particles by flowing electricity"*. Quincke asserted that[10]

> … Reuss in Moskau beobachtete zuerst, im Jahre 1807 daß ein galvanischer Strom Flüssigkeiten in der Richtung des positiven Stromes mit sich fortführte, wenn die Flüssigkeit an einer Stelle durch eine poröse Scheidewand unterbrochen war. Seine Beobachtungen scheinen jedoch bis in die neuste Zeit hinein wenig bekannt geworden zu seyn, so daß oft Porret, der 1816 ganz ähnliche Versuche beschrieben hat, als der Entdecker dieser später auch wohl mit dem Namen „elektrische Endosmose" bezeichneten Erscheinungen angesehen wird…[33]

In this comment Quincke referred to an unpublished lecture entitled "*Indicium de novo hucusque nondum cognito effectu electricitatis galvanicae" ("Notice of a new, hitherto unknown effect of galvanic electricity")* which Reuss held in November 1807 before the Physical-Medical Society, Instituted at the Moscow Imperial University of Letters.[51] It should nevertheless be pointed out, that although the first observation of electroosmosis should be attributed to the 1807 work of Reuss,[34] Porrett wrote his 1816 paper without any knowledge of its existence.

Interestingly, the history about the discovery of electroosmosis and electrophoresis does not end here. The complete story has been reported recently in a carefully researched and informative essay by Christian Biscombe.[52] The author referred to yet another nearly disregarded report by Nicolas Gautherot, which found only a passing mention by Humphry Davy in his Bakerian Lecture,[35] read November 20, 1806 (ref. [53], p. 20). Gautherot was born in 1753 in Is-sur-Tille (Côte-d'Or)**,** France, in a poor family. He became a well-known composer and musician by profession (in 1799 he published a book entitled "*Théorie des sons*"[54]), and an amateur chemist by interest. Fascinated by the newly discovered galvanic electricity (he was member of the *Société galvanique*, founded in 1802), he had carried out some experiments around 1800. One of his very few reports was read by him at March 17, 1801 (*26 ventôse an 9*) at the *classe des sciences de l'Institut des Sciences, Lettres et Arts*, entitled "*Mémoire sur le Galvanisme*"[55] and published in 1801, only one year after Volta´s publication of his pile. We find the relevant part of Gautherot´s experiments on pages 205 and 206 of ref. [55], which reads

> … Voyant que les plaques métalliques sont fortement oxidées lorsque la pile ou la batterie galvanique a été pendant quelque tems soumise aux expériences, j'ai voulu voir d'une manière plus particulière l'influence de l'attouchement des métaux pour la décomposition de l'eau. Pour cet effet, j'ai placé sur les deux côtés opposés d'une plaque carrée de zinc deux petites bandes de carton pour supporter une plaque d'argent de même dimension que celle de zinc. J'ai place une goutte d'eau entre ces plaques, de sorte qu'elle touchait aux deux métaux. En examinant de tems en tems ces plaques, je ne me suis point apperçu, même au bout de soixante-douze heures, d'aucun effet d'oxidation; tandis qu'un autre appareil disposé de même, avec cette seule différence qu'il y avait une légère communication métallique entre les deux plaques, l'oxidation commencait déjà à être sensible au bout seulement de huit minutes. Ici, l'oxide de zinc, quoi que d'une pesanteur spécifique supérieure à celle de l'eau, abandonne le zinc qui est à la partie inférieure, pour adhérer à l'argent en y dessinant le contenu de la goutte d'eau. Si l'on a laissé écouler assez de tems pour que l'oxide de zinc soit plus abondant, une partie seulement adhère à l'argent, et le reste parait former dans l'eau des espèces de grumeaux gélatineux."[36]

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[32] In his textbook from 1909, W. Ostwald summarized the charge of colloidal particles as follows: " … *So erweisen sich bei reinem Wasser als Dispersionsmittel … als negativ: …die meisten dispersen festen Stoffe wie ..., Stärke, Quarz, Feldspat, Ton, Kaolin,…*"; ref. [50] W. Ostwald, *Grundriss der Kolloidchemie*, Theodor Steinkopff, Dresden, **1909**., p. 233 ("*Thus, with pure water as the dispersant … are negative: … most of the disperse solids, such as … starch, quartz, feldspar, clay, kaolin, …").*

[33] "*… Reuss in Moscow observed, in 1807, as first that a galvanic current transports liquids in the direction of the positive current, given that the liquid is divided at one position by a porous membrane. His observations, however, seem to have become little known until very recently, so that often Porret, who in 1816 described very similar experiments, is regarded as the discoverer of these phenomena, later also termed electric endosmosis.*"

[34] A fact deserves to be mentioned which is nearly always ignored in the context of Reuss` investigations about electrokinetic phenomena. It is his collaboration with the Russian scientist Pjotr Ivanovich Strakhov [1757-1813], who held the chair at the newly-created Institute for Experimental Physics at the Moscow University since 1791. Strakhov was well-known – in addition to his research in other fields – for his observations of electrical conductivity of water and earth.

[35] In a single sentence Davy mentioned that "*M. Gautherot has stated, that in a single Galvanic circle of zinc, silver, and water, in an active state, the oxide of zinc formed is attracted by the silver*;"

[36] "*… Seeing that the metal plates are strongly oxidized when the battery or the galvanic battery has been subjected to experiments for some time, I wanted to see in a more particular way the influence of the touching of the metals on the decomposition of the water. For this purpose, I placed two small strips of cardboard on two opposite sides of a square plate of zinc to support a silver plate of the same size as that of zinc. I placed a drop of water between these plates, so that it touched the two metals. In examining these plates at times, I did not perceive, even at the end of seven-*

We interpret and comment this experiment as follows.[37] At first, Gautherot horizontally arranged a zinc and a silver plate of equal size, the former below the latter, both separated by a small dry strip of cardboard as non-conducting spacer. Note that, in contrast to a Voltaic element, the cardboard was neither wetted nor impregnated. Gautherot placed a drop of water between the plates such that the drop touched both metals. Note also that the two plates were not electrically interconnected at this stage of the experiment (notwithstanding that the impure water Gautherot had available was certainly conductive). Gautherot registered that even after seventy-two hours no indications of an oxidation could be seen. This is what one expects because no electric circuit was closed.

In contrast to his first one, in a second experiment Gautherot interconnected the two plates with a metallic conductor. Hence, upon closing the circuit by the metallic connection, Gautherot observed oxidation at the bottom zinc plate within eight minutes and assumed that zinc oxide had been formed.[38] That was the so far expected result of electrolysis.

However, at the same time Gautherot observed the detachment of particles of this newly formed material from the zinc surface and – despite their higher specific weight compared to water – their movement upwards through the water drop and their adherence at the upper silver plate. Later on, only a part of the particles stuck at the silver surface, the rest remained in the water, dispersed as "*gelatinous*" clots. This material was probably the well-known typical voluminous and jellylike precipitate of zinc hydroxide.

We might assume from this experiment that the positively charged zinc hydroxide particles[39] which were formed by anodic oxidation indeed migrated in the electric field towards the cathode. If we accept this as a fact (even though the description of the experimental conditions is somewhat vague), Gautherot indeed was first who observed electrophoresis, prior to von Reuß and Porrett.

About two years later, on November 29, 1803 Nicolas Gautherot died, as reported by Urbain René Thomas Le Bouvier DesMortiers,[57] caused by a shock from an electric battery (see ref. [58]).

SUMMARY

Here we present the first of a series of papers on the history of observations and method development in the field of (capillary) electrophoresis. In this contribution we take a journey at the outset of what we coin as the "*1st epoch of electrophoresis*", which we outline as a period of 125 years between the 1780s and the 1910s. Due to the striking coincidence with the same period of European political history we deliberately choose to borrow the term "*Long 19th Century*" from the British historian Eric Hobsbawm (see footnote 7), who coined it for the time from the French revolution in 1789 till the beginning of the First World war in 1914. It is astounding that in the course of this epoch nearly all fundamental concepts, models, hypotheses, theories and laws concerning the electrically induced motion of charged particles (in electrophoresis) and of the transport of the liquid medium (in electroosmosis) were formulated, derived and became well-known. But it is the singular and even more astonishing characteristic of this epoch that no approach has been undertaken to utilize all this knowledge for the separation of constituents of a mixture.

It has to be recalled that electrophoresis *by itself* is a drift of charged particles – dispersed in a liquid – under the influence of an electric potential difference. The only specific of *capillary* electrophoresis is that the motion takes place within an open narrow tube, but it is still obeying the general laws of electrophoresis. It is further to note that capillary electrophoresis as we know it from the midst of the 20th century, was first performed as early as in the 1860s, albeit not for separation purposes. As the basic principles of electrophoresis, though it was not named as such, came into the focus of research at about 1800, we find it appropriate to include here the history of the general physical and chemical principles on which it is based.

In the years from 1800 to 1816 three electrically-

ty-two hours, any effect of oxidation; while at another apparatus arranged likewise, with the only difference that there was a slight metallic contact between the two plates, the oxidation already began to be sensible after only eight minutes. Here, the oxide of zinc, although of a specific gravity greater than that of water, abandons the zinc from the bottom part, and adheres to the silver by drawing the content of the drop of water. If enough time has elapsed for the oxide of zinc to be more abundant, only a part adheres to silver, and the rest appears to form gelatinous lumps in water."

[37] Please note that the authors use a terminology in the present interpretation and comments which was not known at Gautherot´s time.

[38] It was probably sparingly soluble zinc hydroxide formed by the zinc ions due to anodic oxidation which were released into the solution. The solubility product of $Zn(OH)_2$ is 3.10^{-17} $mol^3.L^{-3}$. The measured concentration of dissolved free Zn^{2+} ions in water at pH between 6 and 7 and ambient temperature is a few hundred $\mu g.mL^{-1}$ (it is lower than that calculated from the solubility product), see ref. [56] G. Dietrich, *Hartinger Handbuch Abwasser- und Recyclingtechnik*, 3rd ed., Karl Hanser Verlag, München, Wien, **2017**..

[39] This is in accordance with the summary of Wolfgang Ostwald in his standard text book about one century later, which we have cited in the context of clay in Reuss´ experiments: "*So erweisen sich bei reinem Wasser als Dispersionsmittel… als positiv: alle Metallhydroxyde*" (ref. [50] W. Ostwald, *Grundriss der Kolloidchemie*, **1909**., p. 233 ("*Thus, with pure water as the dispersant … all metal hydroxides turn out to be positive").*

induced phenomena were observed upon the application of an electric potential difference to a liquid containing charged or chargeable particles: electrolysis at the electrodes, electrophoresis in the liquid dispersion of the charged particles, and its converse phenomenon, electroosmosis (an electrically-induced transport of the liquid relative to charged surfaces). All these discoveries relied on a source of a constant-flow electricity, not on the static electricity known at the time. This new source was provided by the Voltaic pile, which transformed chemical into electrical energy upon a contact between two different metals with a moistened layer of spongy material in between. It was invented by Alessandro Volta, prompted by Galvani´s incorrect theory of an *animal electricity* published in 1791. We find it thus justified that with this context the history of electrophoresis, and that of capillary electrophoresis commenced.

The discovery of electrolysis is attributed to William Nicholson and Anthony Carlisle, who in 1800, while trying to copy Volta´s pile, observed formation of gas bubbles as a result of the decomposition of river water by galvanic electricity. The history of the discoveries of electrophoresis and electroosmosis is far more intricate. Chronologically, electrophoresis was first observed in 1801 by an amateur chemist, Nicolas Gautherot, who observed motion of small particles (probably zinc oxide or hydroxide) formed at a zinc plate towards a silver plate upon connecting the two by a metal conductor. To his misfortune, however, his experiments were almost completely ignored by the scientific community, he was never cited as discoverer of electrophoresis (mentioned only briefly by Davy´s Bakerian lecture in 1806) and died as a result of electric shock from a battery.

In 1807 Ferdinand Frédéric Reuss (Ferdinand Friedrich von Reuß) reported unexpected generation of flow of water within a V-shaped tube covering its bottom part with quartz sand. After closing the circuit, the water level at the one side of the tube raised, whereas that at the other side decreased accordingly. Upon inverting the polarity, the reversed effect of the water levels occurred. Thus, Reuss unequivocally discovered the phenomenon of electroosmosis. In a second experiment, he placed a quartz sand layer above wetted clay in two water-filled tubes, each with wires dipped into the water as poles. Upon connecting the poles to a Voltaic pile he observed movement of clay particles through the sand. During these experiments Reuss inadvertently, yet undoubtedly, observed both electroosmosis and electrophoresis.

Chronologically, Robert Porrett was the tritagonist in the cast of the play about the priority of the discovery of these electrically-induced phenomena. In 1816 Porrett, not aware of any of the previous discoveries, observed a transport of water from one chamber of a divided jar to another chamber through a bladder divider upon connecting the chambers to the poles of a Voltaic pile. Upon publishing his observation in *Annals of Philosophy*, he gained attraction in the scientific community and up until the middle of the 19th century has been regarded as the discoverer of electroosmosis, in contradiction to the historical facts. It is to note that just as Reuss had no knowledge of Gautherot`s prior experiment observing electrophoresis, neither Porrett was aware of Reuss' priority in discovering electroosmosis.

The above experiments revealing the phenomena of electrolysis, electrophoresis and electroosmosis were merely observatory and offered no formulations of hypotheses on their underlying causes. It is thus expectable that the scientific interest that followed in the subsequent years and decades was directed towards their principles and origins. After the discovery of electrolysis the research on the motion of ions[40] was immediately intensified. Attempts at theories about their inseparable connection, which may have led to an understanding of ion migration, and were undertaken between 1800 and the 1830s, will therefore be the subject of Part 2 of the first series of our historical reviews.

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[40] *Ion* is also a term which was not known at that time. Humphry Davy, one of the leading physicist and chemist at his time, described the nature of ions in a Bakerian lecture in 1807 as follows: *The body possessing the positive energy being repelled by positively electrified surfaces, and attracted by negatively electrical surfaces; and the body possessing the negative energy following the contrary order. (ref. [53] H. Davy, Phil. Trans. Roy. Soc. (London)* ***1807****, 97, 1-56., p.33)*

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Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Historical Articles

The Eminent Russian – German Chemist Friedrich Konrad Beilstein (1838-1906) in the Literature between the 19th and 21st Centuries

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Abstract. Friedrich Konrad Beilstein (1838-1906) was one of the most prominent chemists of the second half of the nineteenth century. His life and scientific achievements were described in the literature published between the 19th and 21st centuries in different countries. The purpose of this paper is to familiarize readers with the important events in the life of Beilstein and his research activities, in particular with selected results of his experimental studies. The names of authors of biographical notes or biographies about Beilstein, published in 1890-2018, and literature on his correspondence are given. In addition, a list of his publications is included.

Keyword: F. K. Beilstein, Organic chemistry, Beilstein test, Handbuch der organischen Chemie, Russia, Germany – XIX century.

Citation: Sztejnberg A. (2021) The Eminent Russian German Chemist Friedrich Konrad Beilstein (1838-1906) in the Literature between the 19th and 21st Centuries. *Substantia* 5(1): 135-156. doi: 10.36253/Substantia-1097

Received: Sep 12, 2020

Revised: Nov 30, 2020

Just Accepted Online: Dec 01, 2020

Published: Mar 01, 2021

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

1. THE IMPORTANT EVENTS IN BEILSTEIN'S LIFE

Friedrich Konrad Beilstein (Fyodor Fyodorovich Beil'shtein, Фёдор Фёдорович Бейльштейн) was called a man of high rank in the profession[1] as well as a man of extraordinary erudition and excellent language skills.[2] One hundred and fourteen years have passed since his death, but in that time little has appeared in the literature about this outstanding man. He went down in the history of chemistry as one of the creators of synthesis in organic chemistry, as well as a person who for a long period of his life systematized organic compounds.

Beilstein was born into a family of German emigrants in St. Petersburg on February 5 [according to the Julian calendar (Old Style); Feb. 17, by the Gregorian calendar (New Style), adopted in Russia on February 1, 1918] 1838, and he was the son of Karl Friedrich Beilstein (1809-1865), and Katharina Margarete (née Rutsch) (1818-1883). His grandfather moved to Russia in his youth from the city of Darmstad.[3]

At the age of 14, after study at *Petrischule*,[4] the excellent German school of the Russian capital, he continued his education (September, 1853-1855) at Heidelberg, where his interest in chemistry was inspired and directed by Robert Bunsen (1811-1899).[5] In 1855, he transferred to Munich, where he

listened to lectures of Justus von Liebig (1803-1873) as well as he studied mathematics and physics. In Munich, under Philipp von Jolly (1809-1884) he completed his first experimental investigation *Ueber die Diffusion von Flüssigkeiten* (Concerning the Diffusion of Liquids).[6] In April 1856, he returned to Heidelberg, where he worked under Bunsen until March 1857. Then he went to Göttingen where, under Friedrich Wöhler (1800-1882), he established murexide as the ammonium salt of purpuric acid.[7] In February 1858, at the age of 20, he submitted his thesis *Ueber das Murexid* to the Philosophical Faculty of the University of Göttingen and obtained his doctor's degree in Philosophy.[8] In October 1858 he went to Paris where he worked in the laboratory of Charles-Adolphe Wurtz (1817-1884) in the *École de Médecine* until September 1859. Then he moved to Breslau (now, Wrocław, Poland), where he became the laboratory assistant of Carl Jacob Löwig (1803-1890).

In 1860, he was invited by Wöhler to return to Göttingen, where he spent six years actively engaged in organic chemistry experimental research.[9] In the same year, he attended the first ever International Congress of Chemists held in Karlsruhe (Germany) on 3-5 September. In November, as a private docent at the University of Göttingen, he began to lecture. In 1865, he was appointed extraordinary professor. From this year, together with Hans Hübner (1837-1884) and Rudolph Fittig (1835-1910), he continued editing *Zeitschrift für Chemie* (1865-1871), founded by August Kekulé (1829-1896).[10]

In 1866, at the age of 28, Beilstein was invited to succeed Dmitri Ivanovich Mendeleev (1834-1907) at the Imperial Technological Institute of St. Petersburg, where he subsequently taught for 30 years. His duties included lecturing on general chemistry (inorganic, organic and theoretical), laboratory management and conducting laboratory classes on analytical chemistry. He performed these duties until 1891, when after 25 years of work and approval as an honorary professor, he still worked as a professor until 1896. In that year, professor Mikhail Dmitrievich Lvov (1849-1899) became his successor.[11]

Since 1867, in parallel with his professorship at the Imperial Technological Institute, he lectured in chemistry at the Nikolaev Engineering Academy and was a chemist in the Council of Trade and Manufactures (CTM).[12] In 1868, he was one of the founding members of the *Russkoye Khimicheskoye Obshchestvo* (Russian Chemical Society) at the Saint Petersburg Imperial University. For his scientific work, in particular for his experimental studies, in 1874 he was elevated to the to the degree of Doctor of Chemistry Imperial Moscow University.[13]

Beilstein was appointed official delegate of CTM to almost all International Expositions, starting from Paris in 1867. After visiting an exhibition in Vienna, together with Alexander Kirillovich Krupsky (1845-1911), they wrote a book in Russian called *Factory Chemical Industry of Western European Countries at the Vienna Universal Exhibition of 1873*. It was published in 1874.[14] A year earlier, Beilstein's book entitled *Die chemische Grossindustrie auf der Weltaustellung zu Wien im Jahre 1873* was published in Leipzig.[15]

"Beilstein's international prestige was to a certain extent contributed by his personal qualities: great erudition, interest in social life and excellent knowledge of languages, which allowed him to take an active part in the work of various international congresses, exhibitions, in celebrations of anniversaries. In addition to Russian and German, French and English, Beilstein was fluent in Italian and Swedish." [16]

Beilstein's participation in the scientific celebrations abroad

Beilstein repeatedly was a representative of the Imperial Saint Petersburg Academy of Sciences on anniversaries, e.g. University of Halle (1894). In 1900, he visited Berlin to participate in the conference devoted to the 200th Anniversary of Royal Prussian Academy of Sciences.[17] Figure 1 is a photography made during this celebration.[18] Benjamin Harrow (1888-1970) inserted this photo on the one of first pages of his book entitled *Eminent Chemists of Our Time*. He also wrote that it "showing several eminent chemists was taken at one of the international scientific gatherings." [19]

Photograph was published by Harrow thanks to the kindness of the Dutch chemist Ernst Julius Cohen (1869-1944).[20] From the left to right are standing: the German chemist Albert Ladenburg (1842-1911),[21] the Danish chemist and historian of chemistry Sophus Mads Jørgensen (1837-1914),[22] the Finnish chemist and historian of chemistry Evard Immanuel Hjelt (1855-1921),[23] the German chemist Hans Heinrich Landolt (1831-1910),[24] the German chemist Clemens Alexander Winkler (1838-1904),[25] who discovered germanium in 1886, and the British chemist and historian of chemistry Thomas Edward Thorpe (1845-1925).[26]

To the left of Beilstein, who sat second from the left, was the Dutch chemist Jacobus Henricus van't Hoff (1852-1911),[27] a Nobel Laureate in Chemistry in 1901, and on the right – the Scottish chemist William Ramsay (1852-1916),[28] who discovered of inert gaseous elements in air (neon, argon, krypton, and xenon) and was awarded the Nobel Prize in Chemistry in 1904, the Russian chemist D. I. Mendeleev,[29-30] who discovered the Periodic Law in

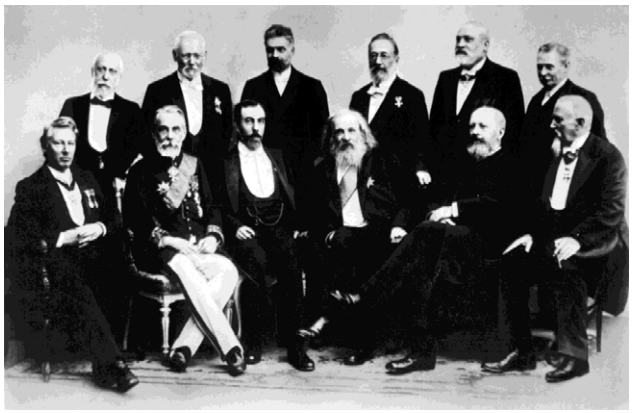

Figure 1. Beilstein with the group of the prominent chemists (Public domain, from reference 18).

1871, the German chemist Adolf von Baeyer (1835-1917),[31] who received the Nobel Prize in Chemistry in 1905, and the Italian chemist Alfonso Cossa (1833-1902).[32]

Death of Beilstein

Beilstein died of a heart attack on October 5 (Old Style); Oct. 18 (New Style) 1906. His funeral took place on October 9 at the Volkov Cemetery in St. Petersburg.[33] In 1945, the Russian-American chemist Vladimir Nikolayevich Ipatieff (1867-1952) wrote in his memoirs: "When I entered the auditorium for a meeting of Phys.-Chem. Society, it has already begun, and the chairman asked everyone to stand up to honor the memory of the deceased. When I asked who died, I was told that Beilstein died on that day... This event struck me very much, especially since F. F. [Fyodor Fyodorovich] was not yet old, full of energy and did not stop working.(…) Belstein was a bachelor, but he adopted one girl, who was the heir to his entire fortune. F. F.'s funeral was organized very solemnly, and a huge number of chemists and other scientists took part in them." [34]

Obituaries have been published in several chemical journals. The Russian chemist Nikolay Nikolayevich Beketov (1827-1911) published his text in *Zhurnal Russkogo fiziko-khimicheskogo obshchestva*[35], and the President of the French Chemical Society, the chemist Armand Gautier (1837-1920) in *Bulletin de la Société Chimique de Paris*.[36] The German chemist Paul Jacobson (1859-1923) wrote about Beilstein in the *Chemiker-Zeitung* in 1906.[37] The German chemist Otto Nikolaus Witt (1853-1915) and E. I. Hjelt published their obituaries in *Berichte der deutschen chemischen Gesellschaft*.[38-39] Witt's obituary also appeared in English in the *Journal of the Chemical Society, Transactions* in 1911.[40]

Otto Lutz published his obituary in *Angewandte Chemie*.[41] One of his statements about Beilstein was also quoted by Lyudmila Anatolyevna Shmulevich and the Russian historian of chemistry Yusuf Suleymanovich Musabekov (1910-1970). They wrote: "Chemistry again suffered a sensitive loss ... Died a man who did a lot for the progress of science, a man who, thanks to his peculiar talent, combined with extraordinary hard work, was able to create a work that has no equal and is intended to facilitate the work of chemists and encourage them to be creative." [42]

2. BEILSTEIN'S WORKS

The list of works published by him includes over one hundred and eighty articles and books that appeared in print for forty-three years from 1856 to 1899. The majority of these are the articles presenting the results of his experimental works, published in the *Zhurnal Russkogo fiziko-khimicheskogo obshchestva* in Russia, as well as in German and French journals. Among them are his original papers devoted to the problems of the of isomerism of the organic compounds, various analytical chemistry issues, and chemistry and technology of the petroleum can be found.

A large number of the results of the experimental research carried out by him were published in German in the *Justus Liebigs Annalen der Chemie, Zeitschrift für Chemie* and *Berichte der deutschen chemischen Gesellschaft*. A few his articles were published in French in the *Comptes Rendus Hebdomadaires Des Séances De L'Académie Des Sciences* and *Bulletin de la Societe chimique de Paris*.

In the period from 1856 to 1882, he published, mainly from organic chemistry, the results of 152 studies. In the next, almost 25-year period from 1882 to 1906, the number of his publications was 27. A large part of all Beilstein's works was done between 1867 and 1884 jointly with few his collaborators, such as Alfons Pavlovich Kuhlberg (1867-1873), Apollon Apollonovich Kurbatow (1874-1883), Ludwig Julievich Jawein (1879-1884) and others.[43]

His first works in the field of organic chemistry was published in 1859 and concerned on the conversion of acetal to acetaldehyde.[44] In subsequent years, aromatic compounds were the main area of his research interests. In 1863, he and Julius Wilbrand (1839-1906) obtained para-nitrobenzoic acid by oxidation of nitrotoluene.[45]

Beilstein's research interests also focused around analytical chemistry. He devised a sensitive test, "the beautifully simple procedure now constantly employed in all

laboratories under the name of the *Beilstein test* for halogens in organic compounds." [46] In 1872, he published it in *Zhurnal Russkogo khimicheskogo obshchestva*[47] and in *Berichte der deutschen chemischen Gesellschaft*.[48] The procedure for the *Beilstein test* was also quoted by an American chemist Eduard Farber (1892-1969) in an article published in *Isis*. Beilstein called the test "not new in principle", since it was based on "the known [the Swedish chemist Jöns Jacob] Berzelius [(1779-1848)] reaction showing the presence of Cl, Br, I in mineral substances by means of copper oxide (...)." [49] Beilstein proposed the following variation: "A little quantity of copper oxide is brought into the ear formed in a platinum wire and fastened there by short heating to a glow. This copper oxide is then dipped into the substance, or a little of the solid material is sprinkled on, and then the wire is brought into a moderately strong flame near its lower and inner rim. At first the carbon burns and the flame is bright, right afterward the characteristic green or blue color of the flame appears." [50]

In 1867, Beilstein's book under the title *Rukovodstvo k kachestvennomu khimicheskomu analizu* (Manual of Qualitative Chemical Analysis) was published in Russia.[51] In the same year, this book was published in Germany under the title *Anleitung zur qualitativen chemischen Analyse*.[52] The second, revised German edition was published in Leipzig in 1870,[53] the seventh in 1892,[54] and the last, ninth in 1909.

The Dutch edition of Beilstein "Rukovodstvo" appeared in 1868.[55] This book has also been published in English by William Collins, Sons, & Company in Glasgow in 1873. The book's translator was William Ramsay.[56] In the United States this book was published in 1876 under the title *An Introduction to Qualitative Chemical Analysis*, translated from the third German edition of Beilstein "Anleitung" by I. J. Osbun.[57] The French edition of this book was published in 1882.[58] He was also the author of a book entitled *Lessons in Qualitative Chemical Analysis* (1883) published in Saint Louis in USA.[59] It was translated from the fifth German edition of his *Anleitung zur qualitativen chemischen Analyse* (1877) by Charles O. Curtman (1829-1896), Professor of Chemistry in the Missouri Medical College. The second edition of this book was published in 1886.[60]

In 1868, he published his *Rukovodstvo k kolichestvennomu analizu* (Manual of Quantitative Analysis).[61] Twenty-two years later, his book written jointly with Jawein under the title *Rukovodstvo k kachestvennomu i kolichestvennomu khimicheskomu analizu* (Manual of Quantitative and Qualitative Chemical Analysis) was published in St. Petersburg.[62] This was the sixth, extended edition of Beilstein "Rukovodstvo" (1867).[63] The seventh edition of this book was released in 1896.[64]

Beilstein is one of the first researchers in the field of chemistry and technology of Russian oil. In the first half of the 1880s, he together with the engineer A. A. Kurbatow (1851-1903) began a systematic study of the composition of the Caucasian Petroleum. Their two important papers on this topic were published in German in *Berichte der deutschen chemischen Gesellschaft* [65-66]. In 1883, their Russian article *Issledovaniye Kavkazskoy Nefti* was also published in *Zhurnal Russkogo fiziko-khmicheskogo obshchestva*.[67] These researchers also showed the difference between Russian and American oil.[68]

From 1881, he continued his scientific activity of other nature. Instead of previous laboratory and experimental work, he began to devote almost all his energy to systematizing organic compounds.[69]

The world's first multi-volume Handbuch der organischen Chemie by Beilstein

The problem of classifying organic compounds according to their properties, methods of preparation, etc., Beilstein found very interesting during his stay in Germany. At the time, he considered writing a handbook to organic chemistry. For almost 17 years of his life, he collected information about organic compounds described in world literature.The fruit of his gigantic and independent work was the first two-volume edition entitled *Handbuch der organischen Chemie*, which was published in 1881 and 1883 in the Publishing House of Leopold Voss in Leipzig.[70] Thanks to his perseverance, it became possible to document and systematize the 15,000 organic compounds[71] known at the time in these two volumes, which comprised a total of 2,201 pages.[72]

After a few years, there was a need to expand and improve this work. Beilstein himself edited the next two editions of his *Handbuch*. The second edition (three volumes, 4,080 pages)[73] was published in 1886-1890,[74-76] and is available in the Internet Archive. The next, third, four-volume edition, which required seven years of his work, comprised of 6,844 pages[77] and contained almost 74,000 organic compounds.[78] It was published in 1893-1899.[79] All volumes of this edition are also available on the Internet.[80-83]

The American chemist and chemical bibliographer Henry Carrington Bolton (1843-1903) was full of admiration for Beilstein's work. He characterized his "Handbuch" as follows: "A stupendous monument of industrious, intelligent compilation." [84] Pavel Ivanovich Walden (1863-1957), Ordinary Academician of the Imperial Saint Petersburg Academy of Sciences, wrote in his *Ocherk istorii khimii v Rossii* (Essay on the History of Chemistry in Russia) that "its significance and fame will survive

many generations of chemists. This is a labor that has been a prototype for other branches of chemical science and a lasting monument to energy, knowledge and diligence of its author."[85]

Supplementary volumes for the third edition of the *Handbuch der organischen Chemie* were published by the *Deutsche Chemische Gesellschaft* in 1901-1906. These were exclusively the work of Paul Jacobson and his collaborators.[86-90]

The Fourth Edition, including five supplements, was published from 1918-1998, covering the chemical literature through 1979. Beilstein realized from as soon as he completed the first edition that a major revision of the classification would be needed. However, he himself never felt that he had the time to complete such a reorganization of the entire work. The "Beilstein System," the basis for the organization of the Fourth Edition, was developed by P. Jacobson, Bernhard Prager (1867-1934), and Dora Stern beginning in 1906. This classification was not subsequently changed; indeed, the stability of the Beilstein System from its origins in 1906 through the final volumes of the fifth supplement to the fourth edition in 1998 was a foundation of great usefulness of the work. The primary challenge necessitating the constant need to publish more and more supplementary volumes was the massive quantity of new research published in organic chemistry, whether it addressed novel compounds or provided additional significant information about previously known compounds.

From 1933, Friedrich Richter (1896-1961) was the director of the Beilstein editorial office and on August 1, 1951, he was appointed president of the Institute in Frankfurt am Main[91] under the name *Beilstein-Institut für Literatur der organischen Chemie* (Beilstein-Institut for literature of organic chemistry), which acted as a non-profit foundation.[92] He was followed by Hans-Günther Boit (1916-1985) in December 1961. Until 2011, the editor of Beilstein *Handbook* was Reiner Luckenbach (1941-2011), who succeeded Boit in 1978[93]. In 1999, the name of the Institute was modified to *Beilstein-Institut zur Förderung der Chemischen Wissenschaften* (Beilstein Institute for the Advancement of Chemical Sciences).[94] In the same year, the Institut "revised and updated its constitution (...), redefining its role in the era of electronic publishing and online information systems."[95]

Richter wrote several articles about Beilstein's *Handbuch der Organischen Chemie*. His first paper was published in *Agnewandte Chemie* in 1925.[96] His book with retrospectives on the *Handbuch* appeared thirty-two years later.[97] His other article under the title Beilsteins *Handbuch – 75 Jahre organisch-chemischer Dokumentation* was also published in *Angavandte Chemie* in 1958.[98] On the hundredth Anniversary of Beilstein's birth, in 1938, Richter published an article in *Journal of Chemical Education*. He wrote in it: "The history of science knows no standstills and famous textbooks mark its course like milestones. When (1881-83), scarcely thirty years after Gmelin's death, Friedrich Konrad Beilstein (1838-1906) put out the first parts of his "Handbuch der organischen Chemie," he could not have foretold that this modest attempt, as he called it, would make his name immortal. From the two small volumes of the first edition it could not have been foreseen that eventually forty volumes would not suffice to house the total treasure trove of organic chemistry."[99]

It should be emphasized here that the German chemist Leopold Gmelin (1788-1853) became very well-known through his *Handbuch der Chemie*, whose provided a model for the kind of reference work that Beilstein compiled. After Gmelin's death, subsequent editions of the *Handbuch der Chemie* had focused on inorganic compounds only, leading to its retitling as *Gmelins Handbuch der anorganischen Chemie*, an inorganic counterpart to Beilsteins *Handbuch* that was also edited by the Deutsche Chemische Gesellschaft beginning in the 1920s.

In 1981, Luckenbach and Josef Sunkel wrote an article titled *Das Wissenschaftliche Handbuch. 100 Jahre Beilstein*, which was published in *Naturwissenschaften*.[100] These authors about Beilstein "Handbuch" wrote: "The Beilstein's Handbook, which in its 4th edition up to the end of 1980 reaches 225 volumes …, covers all organic compounds described in the science literature. Together with "Gmelin's Handbook of Inorganic Chemistry"…, which describes inorganic compounds, they cover almost the entire area of chemistry."[101]

In 1990, the fourth edition of the *Beilstein Handbook of Organic Chemistry* consisted of over 350 printed volumes containing over 275,000 pages of text,[102] and in 1998, reached a total of 503 volumes and over 440,000 pages.[103]

The British historian Evan Hepler-Smith wrote on Beilstein's *Handbuch* and his role in the development of systematic organic nomenclature in his article published in *Ambix* in 2015.[104]

3. BIOGRAPHICAL NOTES OR BIOGRAPHIES ABOUT FRIEDRICH KONRAD BEILSTEIN PUBLISHED IN 1890-2018

In 1890, P. Alekseyev described Beilstein's life and works in Vengerov's Dictionary under the title *Kritiko-Biograficheskiy Slovar' Russkikh Pisateley I Uchenykh (Ot*

Nachala Russkoy Obrazovannosti Do Nashikh Dney).[105] A biographical note about him was published in *Brokgauz – Yefron, Entsiklopedicheskiy Slovar'* in 1891.[106] Seven years later, information about him and his publications appeared in Poggendorff's *Handwörterbuch*.[107] In 1915, Walden[108-109] published his biographical note in *Materialy Dlya Biograficheskogo Slovarya Deystvitel'nykh Chlenov Imperatorskoy Akademii Nauk*.[110]

The Russian historian of chemistry Maks Abramovich Blokh (1882-1941) presented Beilstein's biographical note in his book published in 1931.[111] Richter published two articles about him in German chemical journals in 1938.[112-113] In the same year, Professor of Organic Chemistry at the Massachusetts Institute of Technology (MIT) (U.S.A.) Ernest H. Huntress (1899-1970) published his article about Beilstein's life and works in the *Journal of Chemical Education* in 1938.

The American chemist Henry Monmouth Smith (1868-1950) wrote Beilstein's brief biographie in his book published in New York in 1949,[115] and the British chemist and historian of chemistry James Riddick Partington (1886-1965) wrote about him in his *History of Chemistry* published in 1964.[116] Musabekov and Shmulevich wrote about Beilstein in their article in 1969.[117] His biographical note, written by the German chemist and historian of chemistry Otto Krätz (b. 1937), was published in *Chemie In Unserer Zeit*.[118]

The first in the world Beilstein's full-length biography was written by Shmulevich and Musabekov in 1971. In this book, the first chapter is devoted to the life of the scientist. In the second chapter of this monograph, the authors discussed the experimental research carried out by Beilstein. The third chapter describes his work on the *Handbuch der organischen Chemie*. The structure of the book also includes extensive systematic bibliography and a list of selected literature published until 1969 about Beilstein and his "Handbuch."[119]

A Beilstein's biographical note, written by the Russian chemist Feliks Kazimirovich Velichko (b. 1931), was published in 1972.[120] Luckenbach wrote about him in *Chemie In Unserer Zeit* in 1981.[121]

In 2004, in Göttingen, in the third edition of the publication devoted to the 300th Anniversary of Saint Petersburg appeared an article entitled *Friedrich Konrad Beilstein: Chemiker zweier Nationen* (Friedrich Konrad Beilstein: Chemist from Two Nations).[122] Its author is the chemist and historian of natural sciences Elena Evgenievna Roussanova, candidate of chemical sciences, from St. Petersburg. She works at the *Institut für Geschichte der Naturwissenschaften, Mathematik und Technik* (Institute for the History of Science, Mathemtics and Technology) at the University of Hamburg and at the *Sächsische Akademie der Wissenschaften zu Leipzig* (Saxon Academy of Sciences and Humanities in Leipzig). Two years later, she wrote about Beilstein in an article published in the collection of papers of the International Conference devoted to the 145th Anniversary of the Structure Theory of Organic Compounds of A. M. Butlerov and 100th Anniversary of the Memory to F. F. Beilstein.[123]

The American historian of science Michael D. Gordin, Professor of Modern and Contemporary History at Princeton University, wrote about Beilstein's life and work in his article published in *Chemical Heritage* in 2003,[124] and also in the chapter entitled *Beilstein Unbound: the Pedagogical Unraveling of a Man and His Handbuch* in a book edited by David Kaiser, Professor of the History of Science at MIT, which was published in MIT Press in 2005.[125] Beilstein is also discussed fairly extensively in another Gordin's book that was published by the University of Chicago Press in 2015.[126]

In 2006, a books about Beilstein, written by Roussanova appeared on the 100th Anniversary of his death.[127] One year later, together with Olga Shcherbinina, she wrote an article entitled *Fridrikh Konrad Beyl'shteyn (1838-1906) - K 100 letiyu co dnya smerti russko-nemetskogo khimika* (Friedrich Konrad Beilstein (1838-1906) - On the 100th Anniversary of the Death of a Russian-German chemist).[128] In the same year, Rosussanova wrote an article about Beilstein's election to the Imperial Saint Petersburg Academy of Sciences entitled *F. K. Beilsteins Wahl in die Petersburger Akademie der Wissenschaften*.[129]

Roussanova's article entitled *Friedrich Konrad Beilstein und sein Beitrag zur Kommunikation zwischen Deutschland und Russland auf dem Gebiet der Chemie* (Friedrich Konrad Beilstein and His Contribution to Communication Between Germany and Russia in the Field of Chemistry) was published in 2011.[130] One year later, David E. Lewis, professor of chemistry at the University of Wisconsin-Eau Claire (U.S.A.), briefly described Beilstein's life and selected results of his chemical works in the book entitled *Early Russian Organic Chemists and Their Legacy* in its chapters *Friedrich Konrad (Fyodor Fyodorovich) Beilstein* and *Beilstein's Legacy.*[131] A Beilstein's biographical note, written by R. Klaus Müller, was published in 2014.[132] Roussanova's article under the title *Sankt-Peterburgskiy khimik Fridrikh Konrad Beyl'shteyn* (St. Petersburg Chemist Friedrich Konrad Beilstein) was published in St. Petersburg in 2015 in a book edited by the German historian Dittmar Dahlmann (b. 1949) and the Russian historian Galina Ivanovna Smagina.[133] In 2018, Roussanova wrote about Beilstein's life and works in the first part of her book, entitled *Deutsch-russische Beziehungen in der Che-*

mie des 19. Jahrhunderts (German-Russian Relations in Chemistry in the 19th Century).[134] In the same year, the Academician of the Russian Academy of Sciences, chemist Yuri Alexandrovich Zolotov wrote about him in his book *Ocherki istorii analiticheskoy khimii* (Essays on the History of Analytical Chemistry).[135]

4. LITERATURE ON BEILSTEIN'S CORRESPONDENCE

Ernest H. Huntress, in an article published in the *Journal of Chemical Education* in 1938, provided an English translation of one of Beilstein's unpublished letter to his friend August Kekulé (1829-1896), which nicely exemplifies the 22-year-old Beilstein's rather biting sense of humor.[136]

Otto Krätz is the editor of the collection of correspondence between Beilstein and Emil Erlenmeyer (1825-1909), with whom he maintained close relations.[137] Roussanova's article entitled *Aspekte der deutsch-russischen Wissenschaftsbeziehungen in der Chemie in der zweiten Hälfte des 19. Jahrhunderts in Briefen des Chemikers Friedrich Konrad Beilstein* (Aspects of German-Russian Scientific Relations in Chemistry in the Second Half of the 19th Century in Letters from the chemist Friedrich Konrad Beilstein) was published in 2005.[138] One year later, she wrote an article about new sources of Beilstein's scientific correspondence.[139] Her book, published in 2007, is an edited collection of Beilstein's correspondence, an invaluable resource for those interested in his life, work, and professional and personal relationships with contemporaneous chemists. Besides the already mentioned Kekulé and Erlenmeyer, he was also in a close relationship with Jacob Volhard (1834-1910), as evidenced by his extensive correspondence.[140]

The Russian chemist and historian of chemistry Georgiy Vladimirovich Bykov (1914-1982) was the editor of the book entitled *Pis'ma russkikh khimikov k Butlerovu* (Letters of the Russian Chemists to Butlerow), in which he included letters written, among other, by Beilstein.[141] Together with L. M. Bekassowa he also wrote an article under the title *Beiträge zur Geschichte der Chemie der 60-er Jahre des XIX. Jahrhunderts. F. Beilstein's Briefe an A. M. Butlerow* (Contributions to the History of Chemistry of the Sixties of the XIX. Century. F. Beilstein's Letters to A. M. Butlerov), which appeared in Italy in 1966.[142] At the beginning of the 1880s, Beilstein also corresponded with the Italian chemist Stanislao Cannizzaro (1826-1910).[143-144]

In 1906, Sir Henry E. Roscoe published in his autobiography an English translation of the congratulatory letter he received from his friend Beilstein on the occasion of his Graduation Jubilee on April 22, 1904. A fragment of this letter is as follows:

> HIGHLY HONOURED COLLEAGUE, DEAR FRIEND AND FELLOW-STUDENT, To-day, when so many of your Colleagues, Scholars, and Friends are tendering you their congratulations, you may not be unmindful of the voice of one who is far away, but who has been privileged to follow the development and outcome of your career for fully fifty years. Only a few remain from that happy time, but for that very reason their voices may have the greater weight. It was in the Winter Semester of 1853-54 that I came to Heidelberg to listen, in the class-rooms in the old cloisters, to the teaching of our revered Master, Bunsen, at the end of which Semester you took your Degree. This occurred soon after the beginning of that brilliant period of the academic activity of that immortal man which attracted the enthusiastic youth of the whole world. … In April 1856, when I returned to Heidelberg from Munich, where I had listened to Liebig and worked with Jolly, you and I were colleagues in the new Laboratory, then just finished, and where later on you were a frequent and welcome guest. In June 1856, we—the senior pupils in Bunsen's laboratory—were photographed together. In this picture, which lies before me, I see the forms of many dear old friends who are now no more. Yes, most of them —[Johann Friedrich] Bahr [((1815-1875)], [Ludwig] Carius [(1829-1875)], Kekulé, Lothar Meyer [(1830-1895)], [Leopold von] Pebal (1826-1887)]—have gone. Besides we two, our excellent and honoured comrade Landolt is the only one remaining.[145]

The German chemist Richard Anschütz (1852-1937), professor of chemistry at the University of Bonn in the first volume of his biographical book on Kekulé, introduced the reader to Beilstein's stay in Heidelberg in the second half of the 1850s. He also posted a group photo of young chemists, which Beilstein wrote about in a letter sent to Roscoe.[146-147] Memories of Beilstein's stay in Heidelberg were deeply rooted in his memory, as he also wrote about this photograph in a letter to Anschütz dated 3/16 June 1906, four months before his death. In it he wrote the correct year the photo was taken, compared to the letter he had sent to Roscoe two years earlier. He wrote, among other things: "During my entire year in Heidelberg, I dined with Kekulé daily. We all dined at the 'Darmstadt Hof' (near the end of the main street) and our society consisted of chemists, with the exception of the economist Adolf Wagner [1835-1917]. When I left at Easter 1857 we had ourselves photographed together. You probably saw this picture: in front, in the first row: Kekulé, Pebal, Carius, Bahr, Landolt, behind them: A. Wagner, [Angelo] Pavesi [(1830-1896)], [Agostino] Frapolli [(1824-1903)], Lothar Mayer, Roscoe and others, at the end me."[148-149]

5. CONCLUSION

Friedrich Konrad Beilstein was a great chemist of the second half of the XIX century. In the years 1880-1896, he was elected as a member of two academies of sciences and several scientific associations. He became a corresponding member of the Imperial Saint Petersburg Academy of Sciences on December 3, 1883, and he was elected an Ordinary Academician in Chemistry and Technology on December 13, 1886.[150] He became member of the Academy of Sciences in Göttingen in 1884, the Royal Prussian Academy of Sciences in 1888, and Academy of Science in Uppsala in 1899.[151]

Beilstein was elected as a member of the Royal Scientific Society in Göttingen in 1880. In 1883, he became an honorary member of the London Chemical Society, and two years later – *Deutsche Chemische Gesellschaft* (German Chemical Society). He was elected as an honorary member of the *Société de Médecine de Paris* (Paris Society of Medicine) (1879), the Imperial Technical Society (1888), Philadelphia Medical Society (1893), the Manchester Literary and Philosophical Society (1895) and the Imperial Technological Institute in St. Petersburg (1896).

In 1876, at the proposal of Ordinary Academicians Nikolai Nikolaevich Zinin (1812-1880) [152] and Aleksandr Mikhailovich Butlerov (1828-1886),[153] the Imperial Saint Petersburg Academy of Sciences awarded him the Lomonosov Prize.

Beilstein's death did not go unnoticed. In the years 1890-2018, books and articles with his biographical notes or biographies were published in Russia, Germany, Great Britain and U.S.A. Occasional exhibitions were also organized. On October 26, 2006, the Göttingen Branch of the German Chemical Society organized an exhibition on the 100th anniversary of Beilstein's death at the *Bereichsbibliothek Chemie Niedersächsische Staats- und Universitätsbibliothek Göttingen*. On March 30, 2007, the exhibition was held in the Fundamental Library of the Technological Institute in St. Petersburg, where Beilstein worked from 1866 to 1896.[154]

Since 2005, the *Beilstein-Institut zur Förderung der Chemischen Wissenschaften* has published the *Beilstein Journal of Organic Chemistry* "to provide unrestricted access to high-quality scientific information in the field of organic chemistry."[155]

The name of Beilstein is associated with his *Handbuch* and research in organic synthesis. The results of his experimental studies have been published in scientific journals in Germany, France and Russia. The Imperial Technological Institute where he worked for thirty years was completed by about 1,000 technologists chemists, who, through their work, have made a great contribution to the development of chemical science.[156]

Shmulevich and Musabekov, the authors of Beilstein's biography, wrote about the immortality of his name as follow: "The greatest popularity comes to a scientist when his own name becomes widely known: his name, for example, is called a unit of measurement – and write it without a capital letter – or any edition. In other words, the name is transferred to an inanimate object. This happened with a native of Russia, St. Petersburg academician Fyodor Beilstein, so famous among chemists all over the world. When the word "Beilstein" is spoken, the chemist's imagination is not a man with a thick beard and a handsome, typically learned appearance, but a multivolume handbook, where you can quickly find the information you need about any of the myriad organic compounds."[157]

This outstanding Russian-German chemist took forever a well-defined place in the history of chemistry. His name and the name of Gmelin[158] are associated with the *Gmelin-Beilstein-Denkmünze* (Gmelin–Beilstein Memorial Medal) award. It is awarded biennially by the *Gesellschaft Deutscher Chemiker* (German Chemical Society) since 1954, to domestic and foreign "individuals have produced outstanding contributions to the history of chemistry, chemical literature or chemical information."[159] The prize-winning *Gmelin-Beilstein-Denkmünze* receives a silver medal, certificate and accompanying prize of € 7,500.[160]

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Substantia
An International Journal of the History of Chemistry

Firenze University Press
www.fupress.com/substantia

Book Reviews

Review of *What Is a Chemical Element?* By Eric Scerri and Elena Ghibaudi, eds. Oxford: Oxford University Press, 2020

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OPEN ACCESS

Citation: Kragh H. (2021) Review of *What Is a Chemical Element?* By Eric Scerri and Elena Ghibaudi. *Substantia* 5(1): 157-158. doi: 10.36253/Substantia-1184

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Whereas philosophy of physics and philosophy of biology have for long been well-established academic sub-disciplines, philosophy of chemistry in its modern sense is of more recent origin. The field essentially originated in the late 1980s, not least through the pioneering works and organizational efforts of Eric Scerri. Since then philosophy of chemistry has flourished and attracted much attention not only from historians and philosophers of science but also from some practicing chemists. As indicated by its title, the present work edited by Scerri and Elena Ghibaudi focuses on the nature and meaning of a chemical element, obviously a concept at the very heart of the chemical sciences. The book contains 14 chapters written by specialists in the philosophy and foundation of chemistry, with all of the chapters expertly discussing aspects of what constitutes an element and how the concept has developed through history. Some of the chapters are historically oriented, examining the development from Lavoisier over Dalton to Mendeleev and further on. This is the topic of a penetrating essay by Bernadette Bensaude-Vincent, whereas Nathan Brooks focuses on Mendeleev's ideas prior to his 1869 formulation of the periodic system. Contrary to the common conception, Brooks argues that Mendeleev was not opposed to the idea of elements made up of subatomic units.

Other contributions are primarily of a conceptual and philosophical nature, and others again relate to the history of ideas, such as do the contributions of Farzad Mahootian and Klaus Ruthenberg. While Mahootian discusses the influence of Immanuel Kant and Ernst Cassirer, Ruthenberg calls attention to the non-atomistic ideas of František Wald, Wilhelm Ostwald and Gaston Bachelard. The latter, a French philosopher, introduced the notion of "metachemistry," which to him had quite different connotations than metaphysics. Incidentally, the term is also occasionally used by historians of science to characterize speculative trends in late-nineteenth-century chemistry, such as the views of William Crookes.

A common theme in many of the chapters is the current official definition of an element as given by IUPAC, which defines the concept in a some-

what ambiguous dual form. On the one hand there is the abstract meaning of a species of atoms as given by the number of protons in the nucleus (the atomic number); and on the other hand there is an operational meaning stated in terms of the macroscopic concept of a pure substance. The two meanings are meant to coexist, but do they? As Sarah Nijmans and other authors point out, the IUPAC definition is problematic for both historical and philosophical reasons. While most of the authors are highly critical to the IUPAC formulation, Mahootian suggests that its two parts are not contradictory but rather stand in a complementary relationship. He argues that Niels Bohr's famous but vague principle of complementarity can and should be extended from the realm of quantum physics to elucidate also problems in the philosophy of chemistry. IUPAC's definition of an element reflects to some extent the important ideas of Fritz Paneth, the Austrian radiochemist who in influential works between 1916 and 1962 analyzed in depth the concept of an element. Several of the contributions deal with Paneth's ideas and their later impact, a topic discussed in some detail by Scerri, Hijmans and Joseph Earley, among others.

One of the essays, written by Ghibaudi, Alberto Regis and Ezio Roletto, includes a discussion of the chemical element from an educational perspective, albeit in a rather abstract way which may not be directly relevant to teachers of chemistry. In an interesting chapter on "The Existence of Elements and the Elements of Existence" Robin Hendry focuses on the chemical element from an ontological point of view and with an emphasis on the artificially produced elements at the end of the periodic table. Some of these so-called superheavy elements of atomic numbers $Z > 103$ have been detected only in the form of extremely short-lived atomic nuclei, whereas atoms with their shells of electrons have escaped detection. Nonetheless, they are recognized as proper elements no less real than oxygen and iron. To Hendry, this not only raise questions regarding criteria of existence, it also relates to the problem of the existence of composite bodies in general or what in philosophical circles is known as the "Special Composition Question."

As stated by the editors of *What Is a Chemical Element?* the aim of the book is "to provide an update to the current state of the debate on elements" (p. 2). The book is more than just an update, though, as it offers a series of wide-ranging and in part innovative scholarly analyses of the subject. The attentive reader will not find a final answer to what an element is, but he or she will better appreciate the complexity and many facets of the question.

The book is written by specialists in the philosophy of chemistry and mainly addressed to other specialists, and for this reason it is less relevant to the average chemical reader with an interest in the foundational problems of chemistry. For better or for worse, it illustrates how philosophy of chemistry has come of age as an independent branch of academic philosophy of science. With the independence follows almost inevitably a specialized academic language and a scholarly style that makes the new research area uninviting or even slightly incomprehensible to outsiders. In my opinion, the primary audience of *What Is a Chemical Element?* will primarily be philosophers of science and secondarily historians of chemistry.

Finito di stampare da
Logo s.r.l. – Borgoricco (PD) – Italia

March 2021 No walls. Just bridges

Substantia

An International Journal of the History of Chemistry

Vol. 5 – n. 1

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