The Labyrinthine World of Gregorio Weber

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Abstract The trajectory of Gregorio Weber from his childhood to scientific eminence is examined in the context of the major personages and other influences that he encountered on the way. In the process, unique aspects of his personality, intellect, and philosophical outlook become apparent.

Keywords D'Alessio JT • Gaviola E • Houssay B • Weber number • Yoyo

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1 Getting to Know Gregorio Weber

My first encounter with Gregorio Weber – admittedly indirect – was in 1947. I was 8 years old. Our family flew from Buenos Aires, Argentina, to New York City in a brand new DC4 of Pan American, a journey of almost 2 days. My father, a chemical engineer, was acquainted with a distinguished scientist on board and introduced me to him. His name was Prof. Bernardo Houssay (Fig. 1) and he was on the way to accept the 1947 Nobel Prize in Physiology or Medicine, the first such recognition of scientific excellence in Latin America. In the same year, Houssay's former assistant and protegé, Gregorio Weber (GW [1]), was awarded a PhD in Biochemistry by the faculty of Biology of the University of Cambridge, England.

2 Why This Chapter

Unlike most of the other authors in this volume, I neither studied nor worked nor published with GW, a cardinal oversight on my part. And the experience related above cannot by itself justify my contribution to a book celebrating the centennial of GW's birth. I surmise that Dave Jameson must have felt that enough GW "alumni" and even a relative+scientist (grandniece Cecilia D'Alessio) had passed through – and even survived – our lab (GW visited to make sure), such that *some* relationship of interest must have existed. The alumni were: Francisco ("Pancho") Barrantes, Rob Macgregor, Gerard Marriott, Leonardo ("Leo") Erijman (GW's last postdoc) and his scientist wife Elizabeth ("Eli") Jares-Erijman, who had not worked with GW. Eli came to my lab and Leo to that of Robert ("Bob"), Clegg, a group leader in our Department of Molecular Biology at the Max Planck Institute for Biophysical Chemistry in Göttingen. All of these individuals enriched our scientific and personal lives enormously. Perhaps as a consequence, Bob Clegg underwent a process of "retrograde transfer," at first spending a sabbatical year with GW at the University of Illinois at Urbana-Champaign, and in 1998 accepting a professorial appointment at the urging of Enrico Gratton.[2] In his omniscience Dave may have also recognized the fact that as a "fluorescence fanatic" I belong to the vast community of scientists (and others) who came under the spell of the unique individual we are honoring, and thus can serve as a "prototypic" GW admirer. I have chosen to devote my account primarily to an arbitrary selection of observations, snippets of information, and conjectures seeking to explain how it was that GW developed into the individual who so profoundly influenced our lives. The

[1] I have chosen this condensed form of reference, rather than "Weber," which somehow feels inappropriate.

[2] Eli Jares-Erijman and Bob Clegg, esteemed colleagues, died tragically from cancer in 2011 and 2012, respectively. I have written elsewhere [22] a commemoration of their persons and scientific contributions.

Fig. 1 The three individuals who provided a significant impetus to Gregorio Weber's early career decisions (see text)

treatment has a distinct Argentine bias. Most historical periods and events in GW's career are dealt with in greater detail and accuracy by the fellow authors of this volume, who had a direct, intimate association with GW in the USA and in Argentina (see the chapter of Francisco Barrantes). Dave Jameson has frequently and eloquently narrated GW's scientific legacy in written and oral form [1].

The next, as yet indirect, contact with GW was via Mike Naughton, a coauthor of my first publication in 1964. Mike, a supremely eccentric member of the Biophysics Department of Johns Hopkins Medical School, was an ex associate of Fred Sanger (Nobel Laureate × 2) at Cambridge, and Fred was a close colleague of GW in the 1940s [2]. My first face-to-face encounter with GW and some members of his group was in 1972 – finally! – at a remarkable meeting in Seattle of notables in fluorescence (Fig. 2), who were accompanied by some amateurs like me (as indicated by my position in the photo). By that time, I had been chronically infected with the fluorescence bug (see later section below) and was well aware of the legendary scientist and fellow Argentinian, GW. We hit it off, scientifically and personally.[3]

3 The Historical Brain Drain from Argentina

Let us turn back the clock, to even before 1943, the year in which GW undertook his dangerous exodus to the UK by ship. We probably would not been writing and reading this book were it not for the intervention of certain individuals at key

[3] I communicated with GW in *lunfardo*, the peculiar form of Spanish used particularly in Buenos Aires, in which the second person singular *vos* (and its associated verb forms) replaces the *tu*. It implies intimacy and equality, as opposed to normal Spanish (or English) and "the Professor," which many of GW associates seem to have utilized.

Fig. 2 Participants of the conference *Quantitative Fluorescence Techniques as Applied in Cellular Biology*, Battelle Seattle Research Center. March 27–31, 1972. Names: 1. George Guilbault; 2. Torbiörn Caspersson; 3. Rudolf Rigler; 4. Hans Neurath; 5. Bill Ware; 6. Jane Vanderkooi; 7. Juan Yguerabide; 8. Lenny Brand; 9. George Radda; 10. Gregorio Weber; 11. Ray Chen; 12. Dick Spencer; 13. George Mitchell; 14. Scott Cram; 15. Marv van Dilla; 16. Mack Fulwyler; 17. Jean LePecq; 18. Elli Kohen; 19. Bo Thorell; and 20. Tom Jovin. Spencer and Mitchell were in GW's group at the time

moments in GW's life. It is useful to first consider the circumstances in the country Argentina around the time of his birth. In 1916, the government passed from the hands of the conservatives to the liberal Radical party, initiating a period of somewhat ambiguous social reform and of economic prosperity resulting from massive agricultural exports and the neutrality of Argentina in WW I. In science and education, the start of the century was marked by the consolidation of key developments in the previous 50 years, specifically the creation of: the Department of Exact Sciences at the University of Buenos Aires (1865); the National Academy of Sciences (1869; Charles Darwin was named a corresponding member in 1876); the Scientific Society (1872); and the Astronomical Observatory of Córdoba (1871). Much of this activity was promoted by the prescient, erudite, literary, liberal President Domingo Faustino Sarmiento. The policy was adopted of actively importing brains (professors) and academic research experience, mainly from Europe and also from the USA. Yet after 1900, there was also movement of individuals in the opposite direction, and I cite four cases involving exceptional Argentine scientists (there were more): (Guido Ramón) Enrique Gaviola (1922), Luis Federico Leloir (1936), Gregorio Weber (1943), and César Milstein (1958); the indicated dates are those of their departure from Argentina for graduate or postgraduate work abroad. I will provide further details below but first present some rather remarkable observations that bring GW's experience into perspective:

- Leloir, GW, and Milstein (the first two MDs and both at the urging of Bernardo Houssay) went to the University of Cambridge, and *all three* trained with the enzymologist Malcom Dixon.[4] Milstein received a second PhD from Cambridge, working with Fred Sanger.
- Gaviola, GW, and Milstein made fundamental, pioneering contributions to theoretical, experimental, and applied fluorescence.
- Leloir and Milstein received Nobel Prizes (1970 in Chemistry and 1984 in Physiology or Medicine, respectively). In the estimation of many (myself included), GW was also deserving of such recognition. I don't know if he was ever nominated.
- All four were in opposition to periodic oppressive governmental policies and actions in Argentina and opted for temporary or permanent exile, three of them (Gaviola, Leloir, and GW) to the USA, Milstein to England.[5]

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[4] One can maintain that Malcom Dixon should have been awarded honorary Argentine citizenship for his efforts.

[5] An understanding of the circumstances that have dictated the decision of Argentine scientists to remain (or not) in the country requires a somewhat detailed account. Gaviola, as President of the Argentine Physics Society that he had created, issued a memorandum in 1946 [23] in which he anticipated that because of the postwar political and economic insecurity in Europe and the imposition (tj: *his* perception) of secrecy and censure in the USA, it would be possible to attract first rate scientists to Argentina if they would be provided with the means and the freedom for conducting research and publishing their results without restrictions. GW seems *not* to have perceived the existence of such conditions when he returned for a visit, the first in 4 years, after his PhD award in 1947; his next trip to Argentina was to be many years later. By some accounts, even his mentor Houssay, on the brink of becoming the most important scientific figure in the country, discouraged him from staying, although ambiguity exists about this issue. By 1958, Gaviola himself recognized fundamental deficiencies in the educational system and advocated the creation of private universities in response. GW was a visiting professor/lecturer in the USA in the late 1950s and early 1960s and moved permanently to Illinois in 1962. The fact is that the brain drain of exceptional scientists from Argentina continues to this day, mostly to the USA but

4 Enrique Gaviola

Enrique Gaviola is one of the most influential scientists in Argentine history (Fig. 1). He is recognized as: (1) the first argentine astrophysicist (asteroid 2504 was named after him and asteroid 5987 after Livio Gratton, Enrico Gratton's likewise distinguished astrophysicist father and Gaviola's colleague); (2) an exceptional teacher, also of the general public. He published an article, one of many, in the newspaper *La Prensa* in June, 1930, with what today could be a very topical title (in translation) "The limits of physical knowledge and human vanity. Can we expect an unlimited increase in the accuracy of our measurements with the progress of technology?"; (3) a visionary and activist of and in scientific politics; and (4) a pillar of morality and integrity, including serving as an "Oscar Schindler of scientists" during WW II. Gaviola graduated from the National University of La Plata as a surveyor (!) in 1921 but had developed an interest in mathematics and physics and was thus urged by Richard Gans (one of the "imported" German physicists) to pursue his studies in Germany. He arrived in 1922 to Göttingen, the epicenter of the quantum mechanical revolution. He took courses from the likes of Emmy Noether, James Franck, Adolf Windaus, David Hilbert, Gustav Tammann, Edmund Landau, Richard Courant, Max Born, and Robert Pohl. However, he longed for the big city and transferred to Berlin, where he studied under Albert Einstein, Lise Meitner, Peter Pringsheim, Max von Laue, and Walther Nernst. These lists include 6 Nobel Laureates, and the latter two directed Gaviola's PhD thesis (not bad for a 26-yr-old from Argentina!), awarded in 1926 *magna cum laude* by what is now the Humboldt University. The thesis, entitled (in translation) "The Fluorescence Decay of Dye Solutions," was published in the Ann. der Physik [3] and the novel lifetime apparatus, the "Fluorometer," independently in Z. der Physik [4], a less detailed publication but most often cited as the first experimental demonstration of fluorescence decay measurements *yielding correct results*. Gaviola presented data for the dependence of the lifetimes on temperature, solvent, viscosity, and concentration. He also considered the time lag or "dark-time" quandary of that time, namely the question as to whether if one illuminates a fluorescent substance with a short pulse, it remains dark for a certain finite time before "bursting" into luminosity, which then decreases exponentially. Gaviola concluded that such a dark period does not exist; we will return to this issue below. Gaviola published key papers with his mentor Peter Pringsheim (who also

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increasingly to Europe. A few current, notable cases: Gabriela González (experimental physicist, recent co-discoverer of gravitational waves); Juan Martín Maldacena (theoretical physicist, leading string theory idealogue); and Miguel San Martín (software engineer, responsible for the descent of the rover Curiosity to the surface of Mars). Fortunately, about a decade ago the Argentine government instituted a coherent program ("Raíces," a word meaning "roots" but really the Spanish acronym for the Network of Argentine Scientists Abroad), promoting repatriation of young investigators and instituting significant improvements in scientific infrastructure and support. As a result, Argentine science in 2016 is doing relatively well, and this in spite of the endemic economic (I avoid commenting on the political) vicissitudes.

later ended up in the USA) on questions involving polarization and spent some time with J. Perrin in Paris exploring other issues.

Born and Einstein held Gaviola in great personal and scientific esteem and Einstein helped him obtain a fellowship to the USA. He is reported to have said something like "If only Argentina would have many young scientists like Gaviola..." Perhaps to find out, he spent a month in Argentina in 1925, giving 12 (!) conferences on his relativity theory. The subsequent development of Gaviola's extraordinary career in the USA and later back in Argentina is too extensive for further treatment here (see [5, 6]). I mention only two demonstrations of his productivity. While at Caltech, Gaviola developed the technical means for creating and testing the superior mirror surfaces required for the big reflecting telescopes at the Palomar and Wilson observatories. And although astronomy was his major scientific focus in Argentina, he also initiated in La Plata the construction of fluorescence equipment, including the first fluorimeter in Argentina (to which GW presumably had access), and the production of fluorescent materials in Buenos Aires.

5 The Gaviola–Weber Connection

Why all this attention on Gaviola in a chapter about GW? It appears (at least to me) that as an "earlier edition" he must have had an enormous influence on the scientific directions and development of GW, as well as on his philosophical views regarding the conduct of research and the manner of interaction with colleagues and students.[6] For example, one can assume that GW would have subscribed (or *did* subscribe, judging from his publications and the testimony of his associates) to the "Ten commandments about the scientific method" that Gaviola distributed to his students (my translation):

> 1. Do not steal; 2. Exercise self-criticism; 3. Neither fabricate results nor embellish them by modification of data; 4. Do not practice deception during the exposition of your postulates; 5. Do not conceal information; 6. Do not cease to investigate problems because they may antagonize figures of authority; 7. Do not resort to the invocation of authority; 8. In the execution of an experiment, seek to demonstrate the validity rather than the accuracy of a theory or of a model; 9. In the interpretation of an experimental result, do not exceed the limits of validity of the theory or model in order to achieve better agreement; 10. Do not submit a publication without having first dealt with objections raised by others and yourself.

Furthermore, it is evident from the cumulative writings by and about Houssay, Gaviola, Leloir, and GW (see [1, 2]) that they shared a set of superior human attributes: *intellectual prowess and curiosity; a strong work ethic; a sense of humanity and fair play; generosity and humility; and, by all means not least, a well-developed sense of humor*. GW had an additional, unique ability, rather useful for a research scientist. Shortly before he died, he revealed to his daughter Juliet

[6] For his outlook in 1990, see "Whither Biophysics?" [24].

that he had a perfect memory for everything he had ever read. Others with whom I have spoken appear to corroborate this claim.

6 Family Matters ("Yoyo")

One can identify multiple family and scientific interactions that shaped GW's destiny (see also the chapter of Francisco Barrantes). GW's father, Leon Weber, was an immigrant from Rumania and his mother Rosa, who died of tuberculosis when he was only 7, was a member of the extensive Gerchunoff family, one of the group of early Jewish settlers in Argentina. Her uncle, Alberto Gerchunoff, was a prominent author, columnist, socialist, anti-Nazi activist, and a progressively frustrated assimilationist. There are telling stories about GW in his youth. Within the family and to some of his acquaintances, GW had the nickname "Yoyo" [7] ("yo, yo"="me, me"), bestowed upon him by his older sister Ana Sofía because as a child he was always in the first row, demanding attention (inset, Fig. 3). In later years, GW was more relaxed (Fig. 3) as well as more circumspect in his search for recognition. Ana Sofía was a chemist – she did her thesis on the chemistry of serpents – and married to a chemist–physicist, Juan Tomás D'Alessio (Fig. 1). Both worked in the National Atomic Energy Commission (CNEA), Juan T. being involved in the earliest research with lasers in Argentina. He published interesting and still relevant papers on the production and single/dual photon detection of ns pulses [7, 8], and collaborated with GW in later years on their application in photolysis research and for fluorescence lifetime determinations. D'Alessio's cell biologist granddaughter, Cecilia D'Alessio, stresses the intellectual breadth and generosity of her grandfather. J. T. D'Alessio's son and thus nephew of GW, Enrique D'Alessio, is also a physicist who transformed an academic career (curtailed by the dictatorship instituted exactly 40 years ago) into the commercial design and production of high-end equipment for spectroscopic and automatic clinical analysis. He is very well informed about the orientation of GW's early interests and activities and relates that *it was his father and Gaviola together who prevailed on GW to study physics*.[8] GW's other sister Frida was married to Alberto Kurlat, an extroverted engineer who had a very successful career in the electric power industry and was the only relative to visit GW in England during the 1940s. Considerations regarding the immediate family were key in determining GW's ultimate career decision, i.e. the transfer to the USA in 1962. He had married Shirley Roxana Nixon, a gifted illustrator, and they had three children: Alicia, Rosalind, and Juliet. The unaccountable failure of the academic authorities in

[7] In Argentine Spanish, Yoyo is pronounced a bit like jo in "joke."

[8] In fact, J.T. D'Alessio gave GW classes in physics. Perhaps inhibited by the Cambridge Physics Department of Paul Dirac and others, GW pursued *bio*physics. He did share the view attributed to Dirac that "the laws of nature should be expressed in beautiful equations."

Fig. 3 Gregorio Weber at two stages in his life ("Yoyo," 1923; 1969)

Cambridge and then Sheffield to provide an appointment commensurate with GW's already established scientific stature served to hasten the departure of the Weber family.

7 Why Medicine

We return to the 1940s. It is not altogether obvious why GW studied medicine, although he was in good company, e.g., Luis Leloir, in taking, and probably regretting, this decision. His father Leon was certainly disappointed by the outcome, having established a fully equipped medical consultation room in his home and maintaining it for 20 years in the hope that his son would reestablish himself as a clinician in Argentina. Leloir writes in a brief autobiography [9]: "I was a bad practicing physician because I was never sure of the diagnosis or of the treatment."

GW never got that far and was not exactly affine to the medical profession in other ways.[9] Yet it is interesting to note that the trail from medicine to biophysics is one that has been traversed by many individuals, starting with the Spaniard Nicolás Monardes who in 1565 described the bluish opalescence of the water infusion from the wood of the Mexican tree *Lignum nephriticum*. Ulises Acuña identified the underlying fluorophore 434 years later as the four-ring matlaline [10]; it has a quantum yield of 1! In addition to GW, a historical list, decidedly incomplete, of "fluorescence-biophysical MDs" has other notable entries, 4 of them Nobel Laureates: Paul Ehrlich, Robert Koch, Hermann von Helmholtz, Albert Coons, Elli Kohen, Johann Ploem, Ray Chen, Lubert Stryer, Rudolf Rigler, Sam Latt, Howard Shapiro, and Jens Skou. The undeniable attraction of fluorescence as a *practical* tool in and for medicine, well recognized by GW, is exemplified by the recent contributions of Roger Tsien, a Nobel Laureate but not an MD, to fluorescence-guided surgery [11].

8 PhD Thesis and Beyond

A copy of GW's thesis "The Fluorescence of Riboflavine, Diaphorase and Related Substances" is in front of me as I compose these words. It was written on a typewriter and contains many insertions by hand.[10] GW starts by acknowledging the help of his advisor in building the necessary apparatus but then asserts that "The remainder has been solely my independent work." In the Introduction he makes rather philosophical observations: "I feel that a knowledge as deep of possible, of the physical principles concerned is indispensable. Even a close collaboration with a physicist cannot spare this task to the biochemist." There follows the frequently cited statement to the effect that the brains of a physicist and a biologist working together may come up with 2*n* ideas, few compared to the much larger *n*! of a single brain (tj: presumably GW's) combining both sets of capabilities and knowledge.[11]

[9] It is said that GW had one and only patient, his nephew Enrique D'Alessio, who on one occasion suffered burns from an accident with boiling tea. GW's daughter Juliet relates: "Whenever he (GW) had to see a doctor, he was a terrible patient. Half the time he wouldn't take their advice or the medicines they prescribed." On one occasion, his primary doctor was Tamara Mitchell, wife of George Mitchell, who thus knew him well and recommended he go to a specialist for a certain condition. GW turned the prescribed treatment into a scientific experiment, treating one arm as the target and the other as the control. He was also a reluctant patient during his bout with leukemia. Juliet recalls him saying: "I know what they know and they know nothing."

[10] Richard ("Dick") Spencer, GW's graduate student who created the first cross-correlation phase fluorimeter and was later a co-founder of SLM, did the same in 1970 in a thesis of 308 pages, still a gold mine of information and formalism relevant to excited state dynamics.

[11] Considering that the human brain has ~10^{11} neurons according to current estimates, each with a $10^{4–6}$ synaptic connectivity, he was probably right. Upon reading this footnote, Enrique D'Alessio recalled that Yoyo was a master (tj: as was Dick Feynman) of order of magnitude calculations, which he made during family reunions. One Christmas, someone asked Yoyo how many neurons there were in the brain

The thesis is considered to be a milestone in biochemical fluorescence due to its novel descriptions of the spectral properties of flavins and flavoproteins, including the theory and measurement of emission polarization. However, it is also notable because it has the traces of someone who was still in the process of acquiring the rigor and exactitude that characterized GW's later work. That is, despite the prodigious memory alluded to above, GW demonstrated that he was actually *fallible* (bringing him closer to the rest of us). For example, in the Introduction, and then in thesis Chapter II, GW claims to have experimentally confirmed the existence of a dark period between excitation and emission, a phenomenon that had been postulated more than 20 years earlier, and for which he now provided a formalism based on the viscosity dependence of molecular polarization parameters. This interpretation, however, appears to conflict with the findings and conclusions Gaviola reported in his thesis and then reviewed in 1929 [12], as well as with modern concepts of excited state dynamics.[12] One should note that there were no determinations of lifetimes in the thesis of GW and he relied on the values reported by Gaviola and others, who neither anticipated nor could have resolved multicomponent emission processes. I hasten to state that these comments about the thesis are by no means intended as retrospective criticism, but rather as an indication that GW was operating as a scientific "loner" (compared to Gaviola's situation with a prestigious circle of advisors), and, in fact, continued to function as "his own best postdoc" for a number of years after his degree. Much more can be inferred from a careful inspection of the entire publication record but that is a topic in itself.[13] I restrict myself here to two instances. One is from the only book GW published: "Protein Interactions" [13], which is dedicated to "those who put doubt above belief," a statement akin to some of Gaviola's ten commandments. In the Preface, he wrote:

> At any time and in any scientific subject is comparatively easy to master the concepts that govern what is already understood and widely practiced. It is far more difficult to appreciate that simple concepts, although demonstrably valid in known cases, cannot be extended to

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and he answered (my translation): "Very simple. If we estimate that one acquires one byte of 'knowledge' per second, in 70 years of life this would come to a number $>10^{9\text{–}10}$, and that must be the approximate number of neurons." Whatever the validity of this line of reasoning, the fact is that Yoyo long ago came up with a very respectable value, particularly if one allows, as I would hope, for thinking individuals past the age of 70.

[12] A systematic deviation of an experimental intrinsic anisotropy (r_o) from the expected value can indeed occur due to a variety of reasons (anisotropic motion, solvent relaxation, energy transfer, spectral inhomogeneity, etc.) and is still an issue being addressed from fundamental principles [25].

[13] The story is repeatedly told that the recruitment of GW to Illinois was secured at least in part, by the definition attributed to the department chairman, Irwin Gunsalus, of the *Weber Ratio* = the ratio of outstanding papers to total papers, considered to be unity in the case of GW. I propose generalizing the concept as the function (in *Mathematica* notation) WeberRatio[n_, x_]: = Min [200/n, x], where n is the number of publications and x is the highest value achieved by the target individual during his/her career. For GW, $n = \sim 200$ and $x = 1.0$. I would guess that GW admonished his group to "publish well but neither too little nor too much."

> all systems regardless of complexity. Physical chemistry is an area of science greatly burdened by overconfidence in the universal value of simple rules, and in applying these to the proteins I have tried to make a clear distinction between what we can and cannot take for granted.

In 1960, a remarkable symposium on “Light and Life” was held at Johns Hopkins University. The conference book [14] can be downloaded and is highly recommended. The list of participants is a who’s who of physical and biological science at that time, and included Niels Bohr, who in his Introduction to the symposium made a somewhat less guarded statement than that of GW quoted above: “There appears to be no reason to expect an inherent limitation of the application of elementary physical and chemical concepts to the analysis of biological phenomena.” James Frank introduced GW’s presentation “The excited states of proteins.” GW was asked many questions. One of them had to do with the mechanism by which he had photooxidized tryptophan, and whether he was postulating the intervention of the triplet state. GW rejoined: “No, I was postulating nothing.” He was then asked about the possible existence of excited states of proteins only 1 e.v. above the ground state energy level. GW answered: “I don’t know whether any experiment which I have done on fluorescence of proteins would be related to this question, to tell you the truth. I prefer not to discuss it.” At age 44 but not yet securely established academically, GW was very sure of his strengths and of his limitations.

9 Weber Number

The degree of loyalty to GW’s legacy expressed by his followers 19 years after his death, and well before, is unique. Like in FRET, his influence operated at a distance dictated by scientific and personal “overlaps.” I propose a classification scheme, based on the publication record, which would establish quantitatively and for posterity the connectivity in the Weber scientific family. What comes to mind is a system such as that adopted for Paul Erdős, who ostensibly published more papers than any other mathematician in history. We define a Weber number (Wn) as follows: GW is assigned the number 0 and everyone else has the number $k+1$ with k the lowest Wn of any coauthor of a paper. Thus, those who have published with GW have Wn 1 and those who published with a Wn 1 coauthor have Wn 2. In the list of authors of this volume there are 8 with Wn 1 and 5 with Wn 2. This concept can be extended within the Weber family, for example, to Enrico Gratton, who exhibits a breadth, innovative capacity, and productivity close to that of GW, and deserves his own number, i.e. Wn 1 Gn 0. I hereby claim a Gn 2 but can still strive for a 1.

10 The Influence of GW on my Scientific Career

The most important lessons in life and in science tend to be simple. As an undergraduate at Caltech, I had Richard ("Dick") Feynman as an instructor in sophomore physics (electricity, optics, and magnetism). This was the only time he taught such a course (13 students) and he also served as the lab instructor. He criticized me for having too neat a lab book; he wrote in it: "you can't be writing things down as you work." I stopped using slips of paper for later transcription and the lab book became somewhat more chaotic (I still have it, including his comments). Feynman was satisfied. The take-home lesson, again reminiscent of Gaviola, was: "Record it as it is"; GW would have agreed. The pure and simple approach also guided my later efforts in "serious" fluorescence (and phosphorescence), which had their origins in the lab of Lubert Stryer and Dick Haugland at Stanford. In this case, my mentors were GW and Tomás Hirschfeld, another product of the Río de la Plata but from the other side (Uruguay). Hirschfeld was a prodigious inventor (>100 patents), scientific author (hundreds of papers and notes), experimentalist, theoretician, and entrepreneur, working in numerous areas of spectroscopy, particularly Fourier transform IR but also fluorescence applied in flow cytometry, single molecule detection, and analytical techniques. In a short, seminal paper [15] he demonstrated that the integrated photon output of a fluorophore undergoing photobleaching is independent of quantum yield. This simple, yet not altogether intuitive, observation formed the basis for the introduction by our lab (1989, 1996) of FRET imaging techniques based on donor and/or acceptor photobleaching. Hirschfeld, a gourmand, unfortunately died prematurely at age 48 but has been immortalized by a stamp issued by Uruguay (Argentina could well do the same for GW). He is also remembered for his innumerable aphorisms ("Rules of Thumb"), many, if perhaps not all, of which coincide with known GW philosophical viewpoints. The following is a small selection:

> Persistence at thinking increases average speed and decreases the instantaneous one.
> Remember all phenomena with big derivatives.
> Anything can be made smaller, never mind physics.
> Anything can be made more efficient, never mind thermodynamics.
> Everything will be more expensive, never mind common sense.
> Information theory determines physics, logic supersedes mathematics.
> If the facts don't match your intentions, look for other facts.
> If you do not ask "why this" often enough, somebody will ask "why you."

Earlier in time, the fundamental publications of GW and colleagues on fluorescence polarization had induced us to incorporate emission anisotropy (fluorescence as well as phosphorescence) into home-built cuvette and flow cytometry-sorting systems (1976), and much later (2002) for detecting rotational mobility and FRET homotransfer by phase-modulation FLIM. GW offered invaluable advice during the early developmental phase as well as at the end, when Eli Jares-Erijman and I were elaborating the simple conceptual view of a fluorophore as a photonic "enzyme," turning over input photons (the substrate) into output photons (the product) of lower energy [16]. Determination of the "K_m" from the excitation saturation curve constitutes a measure of quantum yield and thus FRET efficiency. In this FRET imaging review (one of our better cited publications) we stated in the Acknowledgments: "They (we, the authors) are also indebted professionally and personally for the inspiration offered by the late Gregorio Weber, the acknowledged father of fluorescence in biology."

Another main GW theme, environmental sensing by small molecule probes, has been a mainstay of our research over the years. The studies have included the monitoring of amyloid protein aggregation by use of ANS and bis-ANS, both inventions of GW (in fact he gave us the bis-ANS), as well as of novel excited state intramolecular proton transfer (ESIPT) probes [17, 18], some of which are highly solvatochromic [19]. Most recently, we have focused on the development of a range of fluorescent photochromic reagents [20], intended for general use including superresolution microscopy; solvatochromism is also featured. These and the previous studies have involved very able students, postdocs, and visiting scientists, many from Argentina, and benefited greatly from the pioneering publications of GW in this area.

The most recent, current, and as yet unpublished [21], application of "Gaviola +Weber principles" in our lab has to do with the never-ending theme of lifetime determinations in solution and in imaging systems. The decay curve, the emission probability distribution function, is not really of intrinsic interest. Rather, one seeks the underlying rate constants for depopulation of the excited state(s). These we now obtain directly by measuring the lag times (equal to the lifetimes, individual or as population means) between the integrated excitation and emission signals. One avoids the conventional requirement for "delta" excitation by employing long, constant intensity pulses with which a steady-state equilibrium of the excited state is achieved. The "extended excitation FLIM" (eeFLIM) technique is simple, fast, and offers many advantages. GW would have liked it, even if it bypasses the frequency domain, as was the case in our very first FLIM experiments in 1979.

11 Concluding Remarks

There is much more we can derive from GW's intellectual output. He kept copious notes in preparation for a comprehensive book on fluorescence. Dave Jameson is the repository of this material and the scientific community can hope that such a book still comes to be, for our benefit and for the sake of posterity. And before those who knew him personally disappear from the scene, a comprehensive biography should receive high priority. Is it possible to also entice and entrust Dave Jameson with this Boswellian responsibility?

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