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Focal contributions to molecular biophysics and structural biology: a personal view. Part II☆

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1. Introduction

The purpose of this venture is to write a personal history of the biophysical sciences based on focal contributions from approximately the beginning of the 20th century, as seen at almost the end of maybe the most evolutionary, or rather revolutionary, period in the development of the natural sciences. By personal history I emphasize that it is not a complete history as produced by a historian, but rather a live presentation of a selection of scientists, laboratories and events as encountered in the course of a scientific career extending from approximately the middle of the 20th century to the present day [2]. Thus, part of the story is based on personal encounters and recollections, and part on events and personalities close to my own existence, but in contact only through their work or through common personal connectivities. I will, in Part I, give a detailed reason for describing these focal contributions to modern biology. I acknowledge a large number of written recollective contributions, quoted in this work, from which I have culled material for my efforts within the framework, albeit incomplete, of presenting the drama of discovery from various points of view, in a hopefully unified way. I also acknowledge valuable criticism from many highly qualified friends and colleagues who have experienced the same thrill as myself, maybe from a slightly different point of view.

On Thursday October 5, 1995, my wife Nutzi and I were stuck at midnight in a night club in a highly lit entertainment area in Eugene, Oregon. We had flown to Portland and rented a car to drive to Eugene to attend John Schellman's 70 years birthday celebration the following day. We had been given the address of the private home in which we were to be lodged, however, we got lost and were attracted, by the laws of physics and psychology, to the well lit area. We also realized in great dismay that, upon arrival, we had locked the car while leaving our only key stuck inside. The lady at the bar was very nice and arranged for us to call Pete von Hippel, our good friend and organizer of the Schellman celebration. Pete showed up in no time and also produced Deus ex machina his car repairman to extricate the key. So, without more ado and guided by Pete we reached our night destination where our hosts were still waiting for

☆ Dedicated to John Schellman to his approaching 80 years birthday. This work is in three parts with age of dedication decreasing with increasing numbering. Part III has been published ([1]) and Part I has been submitted.

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us. Everything after that went smoothly and we derived great pleasure from this unique event.

John was born on October 24, 1924, in Philadelphia. Schellman's apparently came early from Germany and I found on the web (can we now live without it?) a John Schellman born about 1723 in Germany, married about 1748 to Maria Margareth Fout in Frederick Co. MD and died there on September 25, 1816. 'Our' John completed his Ph.D studies with Walter Kauzmann in Princeton in 1951. In 1953–1955 he was in the Carlsberg Laboratory of Kaj Linderstrøm-Lang in Copenhagen, about which more will be said below, and married Charlotte Green–also a scientist visitor to the Carlsberg Lab–in 1954. They have two daughters, Heidi and Lise. Heidi is a physicist active in neutrino research and I was amazed that, every time I went to the Google—as we all do now whether we like it or not—to collect info concerning John, I was overwhelmed with info concerning Heidi in her manifold physics and related activities. Heidi was a speaker at the Schellman symposium (Fig. 1). Charlotte and John have been active in physical biochemistry studies in Eugene since 1958. They spent 1969–1970 at the Weizmann Institute in Rehovot, creating strong bonds with the laboratories of Shneior Lifson, Ephraim and Aharon Katchalsky, my teacher in polyelectrolyte research. The gist of my article, to get started, will thus be concerned with the Eugene, the Carlsberg and the Weizmann laboratories in their connectivity to John's scientific repertoire. Speakers at the Eugene Schellman celebration were: Buzz Baldwin, Jim Hofrichter, Carlos Bustamante and Pete on macromolecular structure and function; Bengt Norden, Vince Madison and Heidi on waves and particles; and 'historical' talks by Peter Jensen and John, who impressed us with his multimedia presentation of a 'Wayward History of Thermodynamics'. In the evening banquet palaver Rufus Lumry, Howard Schachman and many more contributed to a warm revival of John's and Charlotte's past to the present moment.

2. The Carlsberg Laboratory

The Chemistry Department of the Carlsberg Laboratory in Copenhagen has made outstanding contributions to the physical aspects of protein science over many years, strongly relating to the topics of my present discussion. Much has been written concerning Kaj Linderstrøm-Lang and the Carlsberg Laboratory and I am referring to a small number of pertinent references only [3–6] from where the reader can find access to additional information. The Carlsberg Laboratory was established in 1876 by Jacob Christian Jacobsen, the owner of the Carlsberg Brewery, to perform pure basic research, unrelated to the brewery. The first director of the laboratory was Johan Kjeldahl who developed the procedure for the measurement of total organic nitrogen content of proteins, bearing his name. In 1901 Kjeldahl was succeeded by S.P.L. Sørenson, inventor of the logarithmic pH scale and colorimetric procedures for its measurement, pointing to its relevance to biological function such as enzyme activity. In his studies published in 1917, he established that the ovalbumin protein was a definite molecule, with a molar mass that could be almost correctly determined by osmotic pressure measurements. In August 1919, Lang became an assistant to Sørenson and in 1938 he became head of the Laboratory until his untimely death in 1959. He was strongly influenced by Niels Bjerrum of whom he was a life-long friend. Lang combined in his activities an early correct understanding of the physical chemistry of proteins, an ability to handle the biochemistry of proteins, a capacity to design simple powerful still valid experimental devices, ways of running a laboratory contributing to biology in a friendly, warm and relaxed fashion, combining science with free beer, entertainment, music, painting, figure drawing, side walks to the Tivoli and dense cigar smoke—a rare combination of human endeavor. Of his major contributions to science I would like to mention only that in 1924, less than 1 year after the publication of the Debye–Hückel electrolyte theory, Lang used their formalism to correctly calculate the effect of total protein charge on the apparent pK, the ionization, of titratable groups of proteins, long before information about protein structure was available. In a series of micromethods Lang and his collaborator Heinz Holter turned to the study of biochemistry at the cellular rather

Fig. 1. (a) Heidi Schellman, ladies first; (b) John Schellman; (c) The Schellman Symposium audience.

than the in vitro level. They devised miniature titrimetric methods for the study of small samples. In 1938, Lang and H. Lanz described the gradient tube, a powerful way to measure small differences in density by the creation of a density gradient using two in water immiscible fluids of slightly differing density. He created the concepts of primary, secondary and tertiary structure of proteins and used hydrogen/deuterium exchange under various conditions to study protein structure from a

dynamic point of view, long before nuclear magnetic resonance became available [7].

There is no doubt why in view of the above description of Lang and the Carlsberg Laboratory, young and older scientists from all around the world were attracted to it both before and after World War II. During the war Lang was active in the resistance against the German occupation, and helped slip Jewish refugees and political and scientific personalities, such as Fritz Lipmann [4] and Niels Bohr for instance, out of the country. The fiancé of one of his daughters was arrested with him on the street, and executed by the Germans, while he was luckily released for lack of compelling evidence. He was strongly affected by this event. I have never met Lang myself and have never visited the Carlsberg Laboratory. However, in keeping with the avowed purpose of my presentation, I have introduced its description and unique activities because for so many years it became a center of attraction for so many scientists on short and extended visits, many of whom were close friends of mine in scientific and personal ways. An impressive list of visitors can be found in Schellman and Schellman [6] and I would like to mention here a few, such as Chris Anfinsen, both before and after World War II, Buzz Baldwin, my friend and colleague Arieh Berger [8] from the Weizmann Institute, Bill Harrington [9], Walter Kauzmann, Rufus Lumry, Fred Richards, Charlotte and John Schellman and Harold Scheraga, who figure in my activities in a variety of ways. Charlotte and John, to whom this article is dedicated, spent 3 years at the Carlsberg and were married there, as I have already mentioned. I would like to emphasize two classical 1955 articles by John relating to the peptide bond [10,11] and a joint article on the structural basis of ribonuclease activity, by Chris, Bill, Å. Hvidt, Lang, Martin Ottesen (who headed the Carlsberg Laboratory after Lang's death, 1959–1987) and John [12], with techniques developed by Fred Richards. The paper was recently quoted in the Jubilee Issue of Biophysica Biochimica Acta, with the following commentary [13] by Chris:.....'how an entirely acceptable conclusion can be reached that is entirely wrong because of the paucity of knowledge at that particular time. Indeed I spent the following 15 years or so completely disposing of the conclusion reached in this communication'. Sic transit gloria mundi. However, it is an indication of the strength of science by not being a dogma in the minds and in the hands of its performers.

In the September 1953 issue of the Scientific American, Lang published an article on 'How is a Protein made?', in the dawn of the pathbreaking Watson and Crick Nature article on the structure and predicted function of the DNA double helix.

This concludes my brief discussion of Lang and the Carlsberg Laboratory, whose contributions to the development of molecular biology in the mid-20th century are in line with the achievements of the MRC Laboratory of Molecular Biology in Cambridge, discussed in Part III [1]. However, whereas physical chemistry to biology was the pathway at the Carlsberg, physics to biology shone in Cambridge.

3. The Institute of Molecular Biology, Eugene, Oregon

As mentioned before [1] my first encounter with Terrell Hill was on a visit in 1952 at the Naval Medical Research Institute in Bethesda. We then briefly overlapped in the Yale Chemistry Department in New Haven headed by John G. Kirkwood, while Terrell was working during the tenure of a fellowship on his outstanding book, 'Statistical Mechanics, Principles and Selected Applications', which appeared in 1956. In 1957, Terrell and Laura, strongly attracted to the West Coast, moved to the University of Oregon in Eugene. Terrell was instrumental in attracting a number of outstanding scientists to Eugene and in the creation of the Institute of Molecular Biology. In 1967, the Hills moved to the newly established University of California campus in Santa Cruz, however, in 1971 they returned to Bethesda for science and related activities, such as tennis, for instance, until retirement to Santa Cruz in a full cycle in 1988 [14].

The breadth and the depth of Terrell's work can be appreciated from a listing of his books and monographs in press, in addition to 'Statistical Mechanics: Principles and Selected Applications'

mentioned above. Thus, Barnes and Noble additionally carries: 'An Introduction to Statistical Thermodynamics'; 'Thermodynamics of Small Systems'; 'Cooperative Theory in Biochemistry'; 'Free Energy Transduction and Biochemical Cycle Kinetics'; and 'Linear Aggregation Theory in Cell Biology'. Terrell's elegant contributions moved into statistical non-equilibrium thermodynamics, the sliding filament model of muscle contraction in collaboration with his old friend Manuel Morales as well as with Yi-der Chen and Evan Eisenberg. The understanding of the dynamics of motor proteins from both polymer theory as well as non-equilibrium thermodynamics, converting chemical energy into mechanical work, was approached by Terrell in his book on 'Free Energy Transduction in Biology: Steady-State Thermodynamics Formalism'. Aharon Katchalsky and Paul Flory also pioneered in this field.

Life, however, is not easy. When Terrell moved to Santa Cruz upon retirement he disposed of all his books and papers and decided that from now on he will try to solve mathematical theorems which had engaged him all his life, and now the time had come to concentrate on this. He told me one day some years ago on a visit to Santa Cruz that he proudly sent a proof to a mathematician friend in Boston, and received a reply that his proof is indeed correct, yet it had already been performed in the 17th century. I am confident Terrell has been very successful more recently.

Pete von Hippel was born in 1931 in Göttingen and for the story of his early life I suggest his father's autobiography [15]. In 1933, the von Hippel's left Germany because of the Hitler uprise and arrived in the USA in 1936 after a stay in Turkey and then in Copenhagen, in the laboratory of Niels Bohr. Pete obtained his Ph.D at the MIT in 1955 and collaborated with outstanding scientists in physical biochemistry at the NIH, the US Naval Research Institute in Bethesda, and Dartmouth Medical School. In 1967, he moved to the University of Oregon and in 1969 he became Director of the Institute of Molecular Biology. Pete is known to us all for his wonderful personality and friendship. I remember attending a few years ago a conference on small angle scattering in a residence near Leibnitz, in southern Austria, wandering with Pete and Tom Record for a few hours through the mountains, engaged in scientific and social talk.

Pete's strength in science lies in the combination of a strong physical background with a deep understanding of biological phenomenology. He thus studies the physical chemistry of macromolecules, structure, function and interactions of proteins and nucleic acids and molecular aspects of control of gene expression. In his own words, his group is concerned with the function and regulation of the complexes that control DNA transcription and replication. The transcription cycle is studied both at the overall operon level and at the level of the various steps of the single-nucleotide addition–excision cycle. In the initial stage of the replication work studies concerned the cooperative binding of single-stranded DNA binding protein to single-stranded DNA and RNA, leading to the study of the interactions of DNA polymerase with the primer-template and the polymerase accessory proteins. Going through the complete list I find publications: with D.F. Waugh, his Ph.D thesis adviser, on casein and casein micelles; with Marty Gellert, Manuel Morales and Howie Schachmann on myosin and other muscle proteins; with Bill Harrington, K.-Y. Wong and Elmer Mihalyi on fibrous proteins, gelatin and collagen structure and folding; with S.W. Englander on tritium exchange in the study of protein structure; with Gary Felsenfeld and many more on DNA structure, hydrogen exchange and conformation; with T.W. Schleich and Jim McGhee on salt effects on biological macromolecules and theoretical aspects of DNA–protein interactions; with Arnold Revzin on molecular parameters characterizing the interaction of lac repressor with inducer and non-operator DNA. And so forth and so on, I apologize to collaborators whose names I have not quoted. Finally, a recent compendium on transcriptional control [16].

The Institute of Molecular Biology at the University of Oregon is a typical example emphasizing the physical and chemical background shaping the understanding of the relationships between biological function and the structural properties of the components of living organisms. Sidney Bernhard, born in 1927, completed his Ph.D studies in physical–organic chemistry with Louis P. Hammett at Columbia University, in 1951. His post-doctoral

studies took him to Linus Pauling at Caltech, to the University of Cambridge in England and to the Weizmann Institute in Israel. He worked at the US Naval Medical Research Institute and then at the NIH in Bethesda. In 1961, he joined the Chemistry Department and the Institute of Molecular Biology at the University of Oregon, concentrating on a wide range of topics in physical biochemistry and molecular biology. Sidney died in 1988 at an early age and the breadth and depth of his activities are well described in an obituary written by Michael Dunn and Gian Luigi Rossi [17]. A particular item of interest are the Lighthouse Conferences in Molecular Biology initiated by Sidney in 1975 to provide 'a stimulating environment where established scientists and young investigators could share scientific knowledge and provocative ideas, while enjoying the beauty of the Oregon Coast and the very special home-made seafood dinners that Sidney arranged'. Please read the Obituary for further appreciation.

One of the most exciting papers [18] I have come across combining physical chemistry and molecular biology originates from the laboratory of Jerome Vinograd at Caltech relating to the analysis of sedimentation equilibrium in buoyant density gradients [19–21]. Overshadowing the interest in the unique elegance of sedimentation in a density gradient as a versatile physico-chemical tool, for the investigation of macromolecules so large as to be difficult to investigate by the conventional equilibrium method, is its remarkable usefulness as a biological tool, in particular in the novel way in which it has utilized isotope-labeling methods [20,21]. The classical experiment in this field is due to Meselson and Stahl [18], who showed that the replication of *E. coli* DNA was semi-conservative by following banding patterns of subsequent generations of density labeled [^{15}N]DNA transferred to the light [^{14}N] isotope medium. The isotopically labeled and non-labeled materials were clearly separated in the equilibrium sedimentation pattern. Franklin Stahl was born in Boston in 1929, and received his Ph.D in biology in Rochester University in 1956. He was at Caltech between 1955–1958 and joined the Institute of Molecular Biology in Eugene in 1959 concentrating on research in mechanisms of recombination and bacteriophage genetics.

In our present times, the strong methodologies in structural biology are X-ray crystallography and nuclear magnetic resonance, providing detailed information of structure and mobility of biological macromolecules, advancing our knowledge to a precision and understanding which were not available at an earlier period. We should, however, remember that the biological machine is more complex and an understanding of its activity requires an understanding of both its components and the way they are combined and interact in the operation of the machine. Thus, the invention of the wheel in times prehistoric was a remarkable event and provided the basis for the eventual creation of a variety of simple and complex mechanical machines. However, appreciation and understanding of the powerful symmetry of the circular wheel structure is not sufficient to describe its performance in sophisticated man-made machines. It takes more than the precise description of the broken down components of an automobile on a pile, to reconstruct a gasoline consuming, running down the highway, monster.

Brian Matthews obtained his Ph.D at the University of Adelaide in 1964, post-docked with David Blow at the MRC-LMB in Cambridge and with David Davies at the NIH in Bethesda, and joined the Institute of Molecular Biology in 1969. Brian uses X-ray crystallography, in concert with other techniques, to address some of the fundamental problems in biology: How do proteins spontaneously fold into their biologically active three-dimensional configurations, what determines their stability and can it be improved by changes in composition? How do proteins interact with each other and with DNA, and how do enzymes interact with their substrates and act as catalysts? Brian and coworkers inter alia use the lysozyme from bacteriophage T4 to define the contributions that hydrogen bonds, hydrophobic interactions and salt bridges make to the stability of proteins [22].

The strength of John's studies over many successful years consists in the examination of biological macromolecules, proteins and nucleic acids, in terms of their interactions with large and small molecules, solvents and solutes, sometimes in

'crowded' environments, mimicking biological media. There is strong overlap between his and our own work [23,24] leading to complementary referencing. I would like to sample in mentioning John's outstanding contributions, fluctuations and linkage relations in macromolecular solutions [25], a simple model for solvation in mixed solvents [26], the relation between the free energy of interaction and binding [27] and the thermodynamics of solvent exchange [28].

4. The Weizmann Institute of Science Biophysical Laboratories

Higher education and scientific research was started in Israel (then Palestine) in 1924 with the foundation of the Technion (the Haifa Institute of Technology) and the Hebrew University in Jerusalem in 1925. The early history of biochemistry in Israel has recently been discussed by Nathan Sharon [29]. A small number of dedicated scientists and teachers came from Europe and provided a haven for gifted Israeli (Palestinian!) young students. Aharon Katchalsky was born in 1913 in Lodz, then Russia, and his brother Ephraim Katchalski (this is only a matter of spelling) in 1916 in Kiev, also in Russia. In 1922 the family moved to Palestine. In 1930, Aharon enrolled in the newly-established Faculty of Science of the Hebrew University and eventually completed his Ph.D on the reaction of aldoses with amino acids or peptides under the direction of Max Frankel who headed the section of macromolecular chemistry. Aharon did not further study carbohydrates, however, from 1950 to 1953 he instructed Nathan, an outstanding innovator at the Weizmann Institute in the study of carbohydrate protein interactions, in his Ph.D research [30]. Aharon's research interests turned into polyelectrolytes, as models of biological macromolecules, and I became his student in 1946 upon release from service in the British Army in World War II [2].

Ephraim enrolled in the Hebrew University in 1932 and undertook his Ph.D research with Frankel on the synthesis of polyamino acids, towards an understanding of protein structure and function. This topic, with its manifold ramifications, remained with him in a lifetime of outstanding research performed at the Weizmann Institute from 1949 to the present date. In that year, the Weizmann Institute was opened and I will concentrate on some of its activities in the following [31].

The concept of a polymer, or a macromolecule, is basic in the creation of life, be it on functional or structural grounds, and it is no accident that the description of the DNA double helical structure by Watson and Crick in 1953 also emphasized its fundamental informational nature. It created the science of molecular biology, which has dominated modern science ever since. Aharon, head of the Polymer Department at the Weizmann Institute since its opening in 1949, visualized a class of charge-carrying polymers, polyelectrolytes, broadly significant in both biology and in the physical sciences. The name polyelectrolytes had been coined by Ray Fuoss in 1947, and in 1951 he became our first distinguished scientific visitor, attracted by our initial publications in this exciting new field. Structure in biology is heavily dependent on proteins, carbohydrates and nucleic acids, and diverse aspects of function relate to electrostatic charges spread on these macromolecules. This represented a basic and far reaching thrust into the big unknown and provided tremendous stimulation towards an improved understanding of biological structure and function. Werner Kuhn and Paul Flory had worked out the rules for the behavior of macromolecular chains in solution due to Brownian motion, and could relate these to hydrodynamic and thermodynamic manifestations, and sedimentation in the ultracentrifuge. Alex Silberberg came to us from Kuhn's laboratory and extended his studies on theoretical and experimental aspects of polyelectrolyte behavior. He and his students early on discovered an interesting phenomenon distinguishing polymethacrylic acid, our major polyelectrolyte material in these days, from polyacrylic acid, which had been studied by Werner Kern and Herman Staudinger. When solutions of polymethacrylic acid were stirred rapidly, clear solutions gelled into solid-like material which would recover their liquid form after a while, demonstrating an important structural principle related to hydrophobic interactions deriving from the additional methyl group absent in polyacrylic acid. It was given the name negative thixotropy by the

authors. My 1952 doctoral thesis, guided by Aharon, emphasized the transition from polyelectrolyte solutions to mechano-chemistry, the transformation of chemical energy into mechanical work, following crosslinking of molecular polyelectrolyte chains [32]. This then provided the basis for much of my future work [2,33].

In my postdoctoral studies with Fuoss at Yale University in 1952, I deepened my understanding in electrolyte behavior and statistical mechanics. Indeed, the Chemistry Department at Yale, with Kirkwood, Onsager, Fuoss, Sturtevant, Harned and Owen was the most sophisticated institution then dealing with these problems. Upon returning to the Polymer Department in 1953, I extended my work on simple electrolyte conductance performed during my stay at Yale, to the design of conductance cells useful in the study of ion condensation in polyelectrolyte solutions. The first method which Aharon and Pnina (Spitnik) Elson had actually used in polyelectrolyte research was potentiometric titration. As protons are removed from a polycarboxylic acid, an electrostatic field arises, which increases with increasing dissociation (pH). It becomes increasingly more difficult to remove protons, the acid gets weaker and weaker, and the potentiometric titration of the polyacid is different from that of the simple acid. Aharon's work continued for many years [34] until his untimely death in 1972 [35].

Great ideas in science and other human activities must be viewed in the context of the times in which they were created. In final analysis, specificity and fine-tuning are responsible for the precise functioning, preservation and replication of the biological machine, yet an understanding of the basic principles grafting function on structure cannot be easily dispensed with. Thus, early steps comprised physical analysis in solution, strongly affected by: electrostatic charges; hydrogen bonds; shapes of expanded 'worm-like' coils; conductance; potentiometric behavior; surface interactions in solutions—studied by Israel Miller in the Polymer Department—and suspensions of natural and synthetic macromolecules. Viscosity is strongly shear-dependent in solutions of polyelectrolyte chains expanded by the repulsion of electrostatic charges, and special devices and approaches were created to overcome these problems. Classical rules devised to interpret the behavior of non-ionic macromolecules in the analytical ultracentrifuge, or the scattering of light or of X-rays and neutrons were extended by Ed Casassa and myself, during my stay between 1958 and 1960 at the Mellon Institute in Pittsburgh then headed by Flory, by devising a theory for the thermodynamics of charged synthetic polyelectrolyte and biological multicomponent systems [20,21,23,36]. In the present, renaissance and broad use of the Beckman XLI novel analytical ultracentrifuge this approach is not followed by many for unjustified practical reasoning, although Fujita in his classical text [37] and more recently [38] has pointed out the significance of the Casassa–Eisenberg derivation which should not be disregarded in the analysis of multicomponent biological systems.

It was a well-known and accepted concept in the early days of polyelectrolyte research that viscosity was an important method in the study of polyelectrolyte expansion with increasing charge and decreasing salt concentration. However, polyelectrolyte viscosity was strongly shear-dependent and the use of capillary viscometers was not leading to correct and reliable results. In a joint study with Jean Pouyet, I was exposed to the Vallet Couette viscometer in the Charles Sadron Centre de Recherches sur les Macromolecules in Strasbourg and decided with Ephraim (Heini) Frei, then head of our Electronics Department, to design and build a Couette viscometer with electrostatic restoring force covering a wide range of rates of shear, without using the classical galvanometer suspension wire. We also received valuable advice from our physicist friend Saul Meiboom, who later became one of the early pioneers in NMR research. With the help of the instrument, we could determine viscosities of synthetic polyelectrolytes and of biological macromolecules such as DNA and RNA, correctly extrapolated to zero concentration and zero rate of shear. Valuable information thus became available, as for instance my work with Uriel Littauer showing in 1958 that RNA, unlike

DNA, is essentially a single stranded molecule. Please consult Watts [39] for pertinent references.

Polyelectrolyte chains connected in three-dimensional networks created, as already mentioned, mechano-chemical systems capable of transforming chemical energy reversibly into mechanical work. Though far from leading to a correct interpretation of muscle contraction, which is still a hot topic today, conceptual advances contributed to a better understanding of biological motion [40]. Next, attention veered to irreversible thermodynamics, essential in the maintenance of the processes of life, and in particular to the behavior of biological membranes, in the work of Ora Kedem and Aharon [34,35].

Aharon moved more deeply into the problems of the origin of life—trying to mimic prebiotic synthesis by polymerizing with Mela Paecht amino acids and nucleotides by heterogenous catalysis on mineral surfaces, such as swollen montmorillonite clays—and into the philosophical aspects of science [34,35]. He was killed on May 30, 1972, in the main hall of the Lydda/Tel Aviv airport, together with many other innocent passengers, by a Japanese hired by an Arab terrorist group—returning home from a meeting with Manfred Eigen in Göttingen. The senseless death of Aharon was a heavy blow to Israeli science. However, a broad range of activities continued unabated even though the Department was weakened by the departure of the membrane group to establish an independent Membrane Department, led by Ora, in 1974.

Interests in the Polymer Department continued to range over a wide spectrum, in step with the advancing frontiers of biophysical sciences. A shift of emphasis gradually developed, progressing from the fundamental study of synthetic charge-carrying polyelectrolytes to the investigation of more complex biological macromolecules, enzymes from halophilic bacteria surviving at extremely high saturated salt concentrations in the Dead Sea [39], nucleic acids and chromatin [39]. Our Department became a unique place in the scientific world in which joint ultracentrifuge, elastic and inelastic light scattering, X-ray scattering and neutron scattering experiments, by collaboration with Joe Zaccai in Grenoble, could be undertaken on important biological macromolecular systems [41].

Glycoproteins represent a major secretion of the mucous membrane, the study of which was actively pursued by Alex on a physiological, biochemical and physico-chemical basis. Another related field of their study was concerned with the biorheology of epithelial mucus, blood, fibrin clotting and platelet aggregation.

Pierre-Gilles de Gennes, invited by Shlomo Alexander to Rehovot in 1966 for a Solid State physics meeting, was all excited having quite recently discovered the existence of polymer science and spent many exhilarating afternoons and evenings with Alex, myself and other members of our Department, to strengthen his grip on this fourth state of matter. Later, in his Nobel Lecture, de Gennes stressed the impact of polymer science on biological phenomena. In 1977, Jacob Klein, product of the new Polymer Physics trend, originated by de Gennes and by Sam Edwards, joined the Polymer Department, and extended his novel approach in the evaluation of forces on the Ångstrom scale by synthetic polymers absorbed on mica surfaces in solution. Sam Safran, expert in the theory of colloids and surfaces, came from the USA to join the Department. Jacob Anglister introduced multi-dimensional NMR protein structural studies into Israel. Ed Trifonov, who came from the Soviet Union in 1977, coined the term, now universally accepted, of DNA ‘bendability’, and is expert on manifold aspects of the genetic code, a field he calls Gnomics. In October 1991, the Department was disbanded in a broad organizational move at the Institute and its members were transferred into the newly founded Departments of Structural Biology and Materials and Interfaces.

I am coming back now to work generated by Shneior and others in the Polymer Department on polyelectrolyte theory [40]. Early excitement generated by Shneior in developing theoretical approaches to understand the precise details of polyelectrolyte behavior was dampened by the realization of the enormous difficulties, persisting today, facing the solution of this problem. No good theory was available, and is not available to the present day, for the determination of polyelectrolyte dimensions. Theories based on the expansion

of coils all overestimated the force of repulsion and yielded fully stretched macromolecules, which was not confirmed by simple reliable experiments [32]. Shneior and Aharon started to work on a model which, they felt, though it would not be useful to determine dimensions, would be useful for calculating potentiometric titrations, ion-binding, conductance, osmotic pressure and so forth. This model states that for relatively short distances along the chain it is possible to assume a rod-like shape and calculate the distribution of counter-ions surrounding a cylinder with equally spaced oppositely charged co-ions. This model of polyelectrolyte behavior, later extended by Zeev Alexandrowicz to include simple salts, and known as the cell model, has maintained itself in one form or another to the present day.

In 1963, Shneior left the Polymer Department and established the Department of Chemical Physics. His outstanding achievements in a life of theory in the chemical and biological sciences are well-recorded [40]. He was born in Tel Aviv on March 19, 1914 and continued riding on his bicycle to his office to almost the day of his death on January 23, 2001. He had completed, with his mathematician wife Hanna, a paper on the coexistence and Darwinian selection among replicators, which was submitted after his death and appeared recently [42]. Work on the crucial stages in the origin of animate matter was what occupied Shneior, in recent years, started following his friendship with and inspiration by the work of Manfred Eigen in this field. From his initial studies on polyelectrolyte theory Shneior moved to viscosity in fine capillaries (called 'viscosity in five capillaries' in a particular abstract) and instability of fluid jets in his stay with Peter Debye at Cornell University. He completed his Weizmann Fellowship with J.J. Hermans in Leiden, member of the Dutch School of Polyelectrolytes [43]. This led to the Ising model theory of polyelectrolytes upon his return home, in collaboration with Bruria Kaufman, who had dared to reformulate the Ising model with Lars Onsager. With Julius Jackson, Shneior calculated the effective diffusion constant of ions in a polyelectrolyte solution, and with Irwin Oppenheim the effect of solvent on chain statistics, linking matrices to matrices in 'amusing mathematical acrobatics'. With the mathematician Jack Warga, Shneior developed a theory of 'asymmetric titration' forming 'quasi-grand partition functions' which only later came into good use. During his sabbatical stay at Harvard with Paul Doty, Shneior developed and tested with Carl Schildkraut the dependence of the DNA melting point T_m with concentration of salt. With Antonio Roig, Shneior worked out a theory of polypeptide helix-coil transitions favorably comparing to the theories of Zimm and Bragg and Gibbs and DiMarzio. With Bruno Zimm, Shneior created close interaction in this field. Shneior then created the 'Sequence Generating Function', with Dan Bradley, helpful in the analysis of acridine orange binding to DNA, fine details of the helix coil transition in polypeptides and the temperature dependence of the optical rotation of stiff helical polymers. An additional theoretical approach led Shneior to the 'Consistent Force Field' theory applied to solution of problems relating to macromolecules and smaller organic molecules. Collaborators and students not mentioned so far were Jack Dunitz, Mark Green, Avi Shanzer, Clifford Felder, Mordehai Bixon, Arieh Warshel, Arnold Hagler and Mike Levitt. The question of the origin of life had intrigued Shneior from his early days in science and, as already mentioned, remained so to the end.

In his doctoral studies with Max Frankel, Ephraim Katchalski realized that in order to know something about proteins, he would first need to understand the structure and properties, in the solid state and solution, of various high-molecular weight polypeptides [44]. Following the synthesis of polyglycine and poly-L-alanine, Ephraim came across the 1908 Leuchs anhydride procedure, leading with his first graduate student Itzhac Grossfeld to the synthesis of poly-L-lysine. Robert Woodward and C.M. Schramm at that time as well published the synthesis of protein analogs. That is how Ephraim's life-long research program started. In 1949, he moved to lead the Biophysics Department at the Weizmann Institute, following stays at Columbia University with David Rittenberg, at Brooklyn Poly with Herman Mark, Fred Eirich and Turner Alfrey, and at Harvard University Medical School with Edwin Cohn, John Edsall and Larry Oncley. In the long list of polypeptides synthesized

and related activities, the following were involved among others Michael Sela, Pnina (Spitnik) Elson, Arieh Berger, Avraham Patchornik, Gerry Fasman and Leah Bichowski-Slomnitzki. Extensive studies on the biological and physico-chemical properties of the linear and branched polypeptide chains were followed by the study of immobilized enzymes, polymers as chemical reagents, effects of micro-environment on enzyme activities and determination of distance distribution and conformational fluctuations by non-radioactive energy transfer techniques with Itzhac Steinberg and Elisha Haas.

Meir Wilchek and Edward Bayer, in Ephraim's laboratory, developed the avidin–biotin technology based on the high affinity avidin–biotin interaction [45,46], which has become one of the most powerful tools in basic and applied biochemistry and biotechnology, to the extent that the authors of the procedure are taken for granted and often not even referred to.

The synthesis of polytyrosyl gelatin and the demonstration that it is antigenic, in contrast to the unmodified protein, led in 1960 to the preparation by Michael Sela and his doctoral student Ruth Arnon, of the first fully synthetic antigen. As a result, Michael and coworkers, Ruth, Asher Frensdorf, David Givol, Sarah Fuchs and Israel Schechter set up the independent Department of Chemical Immunology, to probe into the realm of proteins and immunity [47]. Israel Pecht joined the Department following expertise acquired in Eigen's laboratory on the temperature dependence of fast reactions.

Michael, born in 1924 in Tomaszow, Poland, joined the Weizmann Institute in 1950 and synthesized polytyrosine and other polypeptides during his Ph.D thesis work with Ephraim. Tyrosylation of converted gelatin created a potent immunogen studied in the Ph.D thesis work of Ruth. Deep friendship and successful collaboration with Chris Anfinsen resulted from a number of stays by Michael at the National Institutes of Health (NIH) in Bethesda. Michael shared in the ribonuclease investigations of Chris, which led to the postulate and conclusions that the native structure of proteins is determined by the amino acid sequence. Michael also collaborated with Bill Harrington at that time in the Anfinsen Lab, and provided Marshall Nirenberg with a solvent for poly-L-phenylalanine, the first product derived from the breaking of the genetic code, UUU encoding Phe. The solvent had been obtained by a curious accident by Michael and Arieh Berger [47,48] in their polypeptide synthesis work. Immunological work in Michael's laboratory in Rehovot then turned to: synthetic antigens; antibodies; genetic control of immune response; synthetic vaccines; antibodies and their drug conjugates against cancer; and finally, copolymer 1 ('Copaxone'), a specific drug against multiple sclerosis, based on synthetic copolymers of alpha-amino acids, charged positively and resembling in their size the myelin protein.

More work is in progress on a very wide front in the areas discussed, in the hands of a new generation of young and dedicated investigators.

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