WHEN LINUS PAULING MET KARL LANDSTEINER

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Linus Pauling (1901-1994) was one of the giants of 20th Century science. His interests ranged from mathematics to DNA. He remains the only person ever to have received two Nobel Laureates on his own; in 1954 for Chemistry, and in 1962 for Peace. In later life he became obsessed by mega doses of vitamin C as a cure-all, for diseases from the common cold to cancer. Indeed, his wife, Ava Helen, and he took large doses of vitamin C for their own, ultimately fatal, cancers, although Ava did seem to enter remission for a year or so.

Pauling, the oldest of three children, was born on February 28th 1901 at Condon, Oregon. His father, Herman, was the local chemist (pharmacist), whose business was always on a knife-edge. He died shortly after moving to Portland when Linus was 9 years old, leaving his ailing wife struggling to bring up Linus and his two younger sisters. She eventually died of pernicious anaemia in 1926, aged 46. The one bright spot in his childhood was his paternal grandparents' home where the children spent many weekends, where *"the woodstove* was *always warm and the smell of rich German cakes filled the air".* (Linus' grand-parents – Karl and Adelheit – were staunch Lutheran immigrants; their native tongue served Linus well in his later years in Germany). After excelling at school in maths and science, collecting butterflies and minerals, making friends when aged about eleven with a boy with a chemistry set and building a primitive laboratory in the basement of his home, he entered the Oregon Agricultural College (later, Oregon State University) at the age of sixteen. The chemistry department at Oregon was expanding rapidly, due to increasing funds from private industry; a typically American trend. Chemistry research also was boosted due to the closure of the German chemistry industrial market in World War I. These were also the years when atomic structure and the contribution of electrons to chemical bonds were being elucidated.

At the age of 21, he joined the new California Institute of Technology (CalTech) at Pasadena, near Los Angeles, as a "graduate student". This was the best place to learn how the new quantum physics could be applied to studying the chemical bond. X-ray crystallography was to play an essential part in Pauling's work. Discovered in 1911 and developed by, among others, the father and son team of the Braggs at Manchester in England, this is an analysis of the pattern of scattering of X-rays when passed through crystals. Pauling developed a singular analytical approach, which by-passed much of the tedious (in those pre-computer days) mathematics required to analyse those patterns. This involved detailed study of the general principles of quantum mechanics, mathematics, atomic and ionic structure, and enabled him to eliminate the less likely theoretical alternative explanations of molecular structure, as revealed by the defracted X-rays. This enabled Pauling to propose accurate molecular structures particularly quickly. He dignified this analytical process with the name "stochastic", although to others it often seemed more like inspired guesswork.

Helped by others, Pauling developed a new concept of chemical bonding – the shared electron bivalency bond. He also described "resonance bonds" (halfway between the purely covalent, and purely ionic bonds), a particularly useful concept when applied to double bonds, so common in organic chemistry. In this, he was way ahead of other chemists, for whom physics and mathematics were hard to grasp (rather like doctors dealing with statistics). His familiarity with chemistry, and his fluency of language, enabled him to describe complex concepts in ways that helped the chemistry community to come to grips with these new ideas.

As the 1920's turned into the 1930's, Pauling applied X-ray crystallography more ambitiously to proteins. Haemoglobin was a particular favourite, because of its

ease of purification and crystal formation. Haemoglobin crystals, however, were not amenable to X-ray crystallography. The trick, discovered in 1934 by the Cambridge crystallographer (and communist) J D Bernal, was to dissolve the proteins in water. By this time, the economic depression following the Wall Street Crash in 1929, was biting deeply, but John D Rockefeller's Foundation (which he formed in the early 20th Century, after being favourably impressed by the way chemists had solved the "cracking" problem of crude petroleum) was continuing to support research in chemistry, biology and medicine. In early 1936, Pauling gave a series of lectures on haemoglobin at the Rockefeller Institute of Medical Research in New York. After one talk, he received a note from Karl Landsteiner, who had been in the audience. As a consequence they arranged to meet.

Landsteiner explained to Pauling that he was trying to work out how antibodies worked, and, particularly, on how they were so specific. Pauling's interest in protein structure had been stimulated by his interest in different varieties of chemical bond in proteins. He found that protein denaturation could occur in two or more phases. The first phase could be reversed, as he showed in a publication in the proceedings of the National Academy of Science in July 1936. By these and similar studies, Pauling revealed that native proteins are polypeptide chains, folded into unique configurations, held together by hydrogen bonds. Slight heating of protein solutions broke the hydrogen bonds, but that careful processes, allowing "re-naturation", could restore them.

Landsteiner's experiments in the 1930's showed that antibody specificity was very precise. He used a range of man-made chemicals. So how could antibodies tell the differences? And how could the body make an infinite number of antibody molecules, especially to "unnatural antigens"? Pauling didn't know, but he was stimulated to read Landsteiner's latest book of immunology. Landsteiner, who had studied under Emil Fischer, wondered if antigens could be used as probes for antibody chemical structure - and, indeed, *vice versa.*

Landsteiner had already developed a concept of "complementarity of shapes", from the "lock and key" theory originating from Paul Ehrlich. Pauling began to think of antigen/antibody binding in a new way; energy considerations require a close fit for weak energy bonds to zip into place, and the antibody would form around the shape of the antigen. In November 1937, Landsteiner again approached Pauling and gave him more "instruction" in the principles of immunology (he was also looking for a job in California). When they met again in 1939, Pauling's interest received another boost. Landsteiner and Pauling's thought processes, however, did not work in quite the same way. Pauling remembered that Landsteiner would ask *"what do these experimental observations force us to believe about the nature of the world?"* whereas he would ask *"what* is *the most simple and satisfying picture of the world encompassing these observations?"*

One idea to explain varying specificity was the possibility of different ways of folding the same polypeptide chain around different antigens. Although this was to lead to a blind alley, it also lead both people to think about the way antibodies work, and also the bivalency of natural antibodies. Nevertheless, the concept of artificial antibodies was particularly attractive, and Pauling wondered if, by careful partial denaturation, and then renaturation in the presence of a new antigen, the original protein molecule could be moulded into a new "specificity". Even though experiments could not confirm this, Pauling went ahead and published a theoretical paper that, at the time, was extremely popular, as it seemed to answer the question about the simplest structure for an antibody molecule and its formation. He explained that the "glue" holding the antibody to the antigen could be formed from a number of relatively weak chemical bonds, acting together (electrostatic, hydrogen and van der Waal's bonds). This led him into disputatious correspondence with other "quantum mechanically-minded" chemists, who suggested, for example, that identical molecules might stick together because of "quantum mechanical resonance". This

led to the concept of complementary molecular shapes; a new “die-and-coin” relationship, as an explanation of biological specificity of enzymes and immunology. Pauling made a special point of stressing the importance of the concept of molecules making complementary copies of themselves. Years later, this was hailed as a founding principle of the new science of molecular genetics, exemplified by how DNA shows both complementarity and identity when reproducing itself.

In the meantime, and still in the 1930’s, Pauling’s collaborators seem to have succeeded in growing artificial antibodies, using animal globulins and different animal antigens. The great excitement caused controversy, as this could never be confirmed by other laboratories, and, decades later, was thought to have happened because an over-eager technician was cooking the books to please his bosses.

These were the only three documented occasions when Pauling and Landsteiner met. The result was a remarkable collaboration of ideas, resulting in the concepts concerning the nature of the intermolecular binding between antibody and antigen. These concepts are valid to this day. Pauling underestimated, as we can now see, how immunological diversity was generated. (I am reminded of the description in a textbook on the immune system by Hobart & McConnell in 1975; they referred to the “Generator of Diversity” as “GOD”). Pauling’s collaboration with Landsteiner pre-dated the discovery of DNA’s double helix by more than a decade.

Molecular biology and genetics, including the genetics of immunity, could not really start until DNA, and the cellular mechanisms of protein synthesis, was better understood. Interestingly, Linus Pauling was on the verge of anticipating Watson and Crick in the concept of the double helix in the early 1950’s. He was helped, to some extent, by the presence of his son, Peter Pauling, in Watson and Crick’s own laboratory at Cambridge. Watson’s own popular account of this historic episode was severely criticised by Pauling and others; not least his criticisms of Rosalind Franklin, whose own X-ray pictures of DNA were instrumental in their analysis. Franklin died before their Nobel Prize was awarded, so was never given the public accolade which many felt, and still feel, she deserved.

Nevertheless, this contact between the ageing and aristocratic Landsteiner, and the still relatively young and brash Pauling, in the mid 1930’s, did bear fruit, and Pauling clearly valued his contact with the “Founder of Immunology”. Interestingly, there is little comment recorded from Pauling when Kohler and Milstein described the manufacture of monoclonal antibodies in the mid 1960’s, although, by this time, Pauling’s work was diverted elsewhere, and he and later collaborators used monoclonals in their work on vitamin C and atherosis. Somewhere his remarks on first hearing about Kohler and Milstein’s work will be recorded – I would like to know what they were!

Much of the information in this article comes from the biography of Pauling, “Force of Nature” by Thomas Hager, Simon & Schuster, 1995.