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Template Theories, the Rule of Parsimony, and Disregard for Irreproducibility—The Example of Linus Pauling's Research on Antibody Formation

ABSTRACT

In 1940, Linus Pauling proposed his template theory of antibody formation, one of many such theories that rejected Paul Ehrlich's selective theory of preformed "receptors" (antibodies), assuming instead a direct molding of antibody shapes onto that of the antigen. Pauling believed that protein shapes—independently of amino acid sequences—determined antibody specificity and biological specificity in general. His theory was informed by his pioneering work on protein structure, and it was inspired by the intuitive "rule of parsimony" and simplicity. In 1942, Pauling published his alleged success in producing specific artificial antibodies through experiments based on his 1940 theory. However, his experiments could not be reproduced by prominent immunochemists at the time, and, later, it became generally accepted that antibody specificity was not generated according to Pauling's and others' "instruction" template theories. A citation analysis shows that Pauling's papers on antibody generation continue to be cited as, among other things, pioneering studies of a chemical technology called "molecular imprinting."

The examples of Pauling and other protein chemists are used in this paper to demonstrate that scientific belief, philosophical concepts, and subjective theory preferences facilitated the occurrence of irreproducibility in immunochemistry and beyond. The article points to long-term consequences for the scientific community if irreproducible results are not acknowledged. It concludes by arguing that despite the risks, e.g., for the occurrence and perpetuation of irreproducible results that they entail, subjectivity and a commitment to scientific convictions have often been pre-

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The following abbreviations are used: IDP, intrinsically disordered protein; IDR, intrinsically disordered regions; MI, molecular imprinting; MIP, Molecularly Imprinted Polymers.

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requisites for the generation, and holding on to, scientific innovation in the face of doubt and rejection from the scientific community.

KEY WORDS: Linus Pauling, antibody formation, irreproducibility, scientific belief, aperiodicity, sequence, molecular imprinting, template vs. selective theories

1. INTRODUCTION

This paper reviews and analyzes scientific developments, philosophical arguments, and the role of scientific personalities in early immunochemistry. The focus is on Linus Pauling and his research on artificial antibody formation in the 1940s. My goals are (i) to analyze the scientific and philosophical rationales as well as personal convictions and characteristics for the choice of different theories and the response to failure, (ii) to propose a novel understanding of important concepts in the history of immunology, such as template and selection, and (iii) to demonstrate the importance of historical research, including an analysis of scientific criticism in the past, to research today.

Linus Pauling (1901–1994) was a highly renowned American chemist who rebuilt chemistry on the new foundation of quantum physics. He contributed decisively to the theory of the chemical bond, and provided the first scale of electronegativity of the elements. Linus Pauling and John C. Slater were instrumental in developing the "valence bond theory," based on the concept of the electron-pair bond,[1] and the concept of "resonance," according to which the normal state of a molecule is represented not by a single valence-bond structure but by a combination of several alternative distinct structures—two ideas that formed the theoretical backbone of Pauling's work. In later years, these theories were largely replaced by R. S. Mulliken's molecular orbital theory, which provided a deeper understanding of chemical bonding. However, Pauling never ceased advocating his valence bond theory, which continued to be used, for example, to explain the planarity of the peptide bond in proteins.[2] His 1939 textbook *The Nature of the Chemical Bond* "taught a couple of generations of chemists that the sizes and electrical charges of atoms determine

1. In this theory a bond between two atoms is formed when two atomic orbitals, one from each atom, merge with one another.

2. Max Perutz, "Linus Pauling, Obituary," *Nature Structural Biology* 1 (1994): 667–71.

exactly their arrangement in molecules";[3] and it later proved essential for the elucidation of the structure of DNA and proteins.

During the late 1920s and '30s, Pauling devised new ways of discovering the molecular structure of complex substances in particular proteins. Three central concepts guided this work: quantum mechanics, which can be used to describe and predict atomic bonding; the structures of simple molecules, which can be used in a building-block fashion to predict the structures of more complex ones; and perhaps most importantly, chemical structure, which can be used to predict chemical behavior. In 1951, Pauling discovered two secondary structures, the α helix and the β sheet, as stable conformations of polypeptide chains. Pauling was awarded the 1954 Nobel Prize in chemistry for "research into the nature of the chemical bond and its application to the elucidation of complex substances."[4]

Pauling played a major role in giving the idea of biological specificity a molecular basis. Biological specificity means that individual organisms, species, and higher entities in the hierarchy of taxonomic ranks (genera, orders, classes, etc.) are unique, and thus different from other entities of the same rank. The concept of biological specificity, which originated in ideas of constant differences between groups of organisms (i.e., *specificity*) in ancient times, has changed over time. In the late nineteenth century, it referred to body structures and proteins that are specific to organisms, species, and so on, and is now explained by the existence of specific information encoded in the genome. Since its beginnings in the nineteenth century, the concept of biological specificity has played a crucial role in biology as a modern experimental science.[5]

The understanding that "differences in the constitution of proteins determine the species specificity"[6] has existed since the early twentieth century, though it was Pauling who in the 1930s advocated the notion that biological specificity manifested itself in the diversity of proteins' three-dimensional structures. According to Pauling, proteins obtained their structure by being

3. Horace Freeland Judson, *The Eighth Day of Creation: Makers of the Revolution in Biology* (New York: Cold Spring Harbor Laboratory Press, 1979), 57.

4. "The Nobel Prize in Chemistry 1954," https://www.nobelprize.org/prizes/chemistry/1954/summary/ (accessed Jul 2021).

5. Ute Deichmann, "Hierarchy, Determinism, and Specificity in Theories of Development and Evolution," *History and Philosophy of the Life Sciences* 39, no. 4 (2017): 3–16.

6. Jacques Loeb, *The Organism as a Whole from a Physicochemical Viewpoint (*New York and London: Putnam's Sons, 1916).

molded to templates, i.e., the surfaces of other proteins, antigens, or genes. As he expressed in 1937: "The secret of life itself is how a protein molecule is able to form, out of an amorphous substrate, new protein molecules that are made after its own image."[7] Focusing on the spatial structure of proteins at a time when its dependence on the amino acid sequence was not yet known, he considered the amino acid composition or sequence not relevant for this structure.

Pauling was known not only for having persistently pursued what became his major achievements, but also for holding on to ideas that turned out to be questionable or mistaken. Best known in this respect is his unsubstantiated and even dangerous claim about the beneficial effects of very high doses of vitamin C.[8] His student Max Perutz, who shared the 1962 Nobel Prize in chemistry for his studies of the structure of hemoglobin, believes that Pauling's preoccupation with this vitamin, which "spoilt his great reputation as a chemist," might be related to "his greatest failing, his vanity." According to Perutz, Pauling "would never admit that he might have been wrong."[9] Another example of Pauling's not giving up ideas that could not be corroborated is his 1940 theory of antibody formation, along with his subsequent claim, in 1942, to have produced artificial antibodies.

In the following section, I shall present Pauling's 1940 theory of antibody formation, its scientific background, and the experimental claims of having produced specific antibodies *de novo*, based on this theory. Though his experiments were irreproducible, Pauling did not comply with the urging of colleagues to disavow them. My analysis of the responses to Pauling's work on antibody formation shows, surprisingly, that this work not only continues to be cited in the field of protein chemistry, but also is regarded as pioneering the technology of molecular imprinting in chemical technology. I use the example of Pauling's antibody research to examine the role of philosophy and of subjective scientific belief in the questionable work of an outstanding scientist, and scrutinize the meaning of irreproducibility in protein chemistry.

7. Archival material in Bruno J. Strasser, "Sickle Cell Anemia, a Molecular Disease," *Science*, 286, no. 5444 (1999): 1488–90.

8. For the creation of and critical responses to this contention, see Thomas Hager, *The Life of Linus Pauling* (New York, London: Simon and Schuster, 1995), 573–97.

9. Perutz, "Linus Pauling" (ref. 2).

2. LINUS PAULING'S 1940 THEORY OF ANTIBODY FORMATION IN THE CONTEXT OF SELECTION THEORIES AND TEMPLATE THEORIES OF ANTIBODY GENERATION IN IMMUNOCHEMISTRY, 1890-1960

2.1. Theories of Antibody Formation before 1940

The histories of immunochemistry in general and of theories of antibody generation in particular have been analyzed in many studies,[10] and I here mention only those aspects that are relevant to my topic. In 1890, Emil von Behring and Kitasato Shibasaburō discovered "antitoxins," which are now known to consist of antibodies, in the blood serum of animals that had acquired passive immunity against infectious diseases. Shortly thereafter, immunochemistry was founded by Paul Ehrlich; his "side-chain theory" of antitoxin (antibody) production for the first time put the focus of research on antigen, antibody, and complement (small proteins enhancing the function of antibodies) as being real molecules.[11] In Ehrlich's selective theory, preformed cellular protoplasmic groups, which he first called "side-chains" and later receptors or antibodies, were selected by toxins (a term that was later replaced by "antigens"). Ehrlich's concept was based on chemical experimentation and his knowledge of the chemical specificity of proteins. He formulated this "side-chain" concept using the broad terms that were available at the time.

At the beginning of the twentieth century, this theory was contradicted by a succession of hypotheses that replaced selection with the idea that antibody specificity is generated by a direct modification of the structure of a normal serum protein by an antigen. The antigen here does not select preformed antibodies, but "instructs" serum proteins to become specific to them. The earliest of such "instructive theories" of antibody formation was proposed by

10. I mention in particular the detailed historical study by Arthur M. Silverstein, *A History of Immunology* (London and New York: Academic Press [1989] 2009), and Thomas Soderqvist's biography of Niels Jerne: *Science as Autobiography: The Troubled Life of Niels Jerne* (New Haven, CT: Yale University Press, 2003); see also Joseph S. Fruton, *Molecules and Life: Historical Essays on the Interplay of Chemistry and Biology* (New York: Wiley-Interscience, 1972); Michel Morange, "What History Tells Us XXXIV: The Complex History of the Selective Model of Antibody Formation," *Journal of Biosciences* 39, no. 3 (2014): 347–50; Thomas Soderqvist, "Darwinian Overtones: Niels K. Jerne and the Origin of the Selection Theory of Antibody Formation," *Journal of the History of Biology* 27, no. 3 (1994), 481–529.

11. Arthur M. Silverstein, "The Historical Origins of Modern Immunology," in *Immunology: The Making of a Modern Science*, ed. Richard Gallagher, Jean Gilder, Gustav J.V. Nossal, and Gaetano Salvatore (London: Academic Press, 1995) 5–20, on 11–13.

Oskar Bail at the German University of Prague before the First World War. According to immunologist and historian of science Arthur M. Silverstein, the theory "would be little improved upon over the next forty years," though Bail's contribution was widely neglected.[12]

By 1930, it was understood that antibodies were globular proteins, and that proteins were somehow comprised of random arrangements of around twenty different amino acids. Based upon the assumption that organisms cannot host specific antibodies against a vast amount of different antigenic determinants, a wave of new "instruction" theories was proposed. Since "instruction" now had to take place "on the level of protein synthesis," for the first time "the notion of antigen-as-template became explicit."[13] Silverstein distinguishes between direct template theories in which antigens directly "instruct" the shape of proteins and indirect ones in which they do so via the modification of other molecules. Most of the template theories discussed here are direct template theories.

The new theory was advanced almost simultaneously by William W. C. Topley, by Friedrich Breinl and Felix Haurowitz, and independently, also by Stuart Mudd and by J. Alexander. Breinl and Haurowitz's proposition that an antigen would be carried in the body to the site of protein formation, where it would serve as a template upon which the nascent antibody molecule might be constructed, became most widely cited. They suggested a stereochemical fit between antibody and the surface of the targeted antigen, i.e., the structural complementarity of the two molecules. As Silverstein emphasized, the theory implied that "the antigen persist[s] throughout the course of antibody formation."[14] The "instruction" template concept of antibody formation became widely accepted among immunochemists.

2.2. Linus Pauling's 1940 Template Theory of Antibody Formation

Pauling entered the field of immunochemistry when immunochemist and Nobel Laureate (for his work on human blood groups) Karl Landsteiner, who in 1936 attended a lecture by him on hemoglobin, asked him how he would explain the observed properties of antibodies and antigens by means of

12. Silverstein, *A History* (ref. 10), 50.
13. Ibid., 52.
14. Ibid.

molecular structure.[15] Pauling started to apply the concept of weak forces such as hydrogen and van der Waals bonds and electrostatic attractions to macromolecular interactions. In 1940, he published a paper with physicist-turned-molecular-biologist Max Delbrück in which they contradicted physicist Pascual Jordan's claim that two identical macromolecules attract each other by quantum mechanical resonance phenomena.[16] Instead, they promoted the idea of complementarity[17] between molecules brought about by weak forces. They perceived the two complementary surfaces like "die and coin," and held that "complementariness should be given primary consideration in the discussion of the specific attractions between molecules and the enzymatic synthesis in molecules."

That same year, Pauling introduced the concept of complementarity into his theory of antibody generation to provide an answer to Landsteiner's question. This theory, which he published in 1940, was one of the latest template theories of antibody formation, and despite its shortcomings (see Section 3.1), it was chemically the most advanced one as it was based on the then latest developments in structural chemistry.[18] Pauling stated, similarly to other template theories, that "an antibody to [a particular] antigen is a molecule with a configuration which is complementary to that of a portion of the antigen molecule," acknowledging that "the idea of complementary structures for antibody and antigen" was first suggested by Breinl and Haurowitz in 1930, then Alexander in 1931, then Mudd in 1932. Applying the concept of weak forces within and between molecules[19] to the interaction of antibody and antigen, he demanded that the "well-defined properties of native proteins require that their molecules have definite configurations, the polypeptide chain or chains in a molecule being coiled in a definite way and held in position by forces acting between parts of the chains."[20] Of particular importance for protein structures were hydrogen bonds, the knowledge of the properties of which had "increased to such an extent during the [previous] five years as to

15. Linus Pauling, "Molecular Basis of Biological Specificity," in *The Double Helix*, ed. Gunther S. Stent (New York: Norton and Co. 1974), 146–53; Hager, *The Life* (ref. 8), 235–36.

16. Linus Pauling and Max Delbrück, "The Nature of the Intermolecular Forces Operative in Biological Processes," *Science* 92 (1940): 77–79; see also Judson, *The Eighth Day* (ref. 3), 117.

17. They called it "complementariness" in order to distinguish it from Niels Bohr's complementarity principle in physics.

18. Linus Pauling, "A Theory of the Structure and Process of Formation of Antibodies," *Journal of the American Chemical Society* 62, no. 10 (1940): 2643–57.

19. Pauling and Alfred Mirsky had used these forces already in 1936 to explain the tertiary structure and denaturation of proteins.

20. Pauling, "A Theory" (ref. 18).

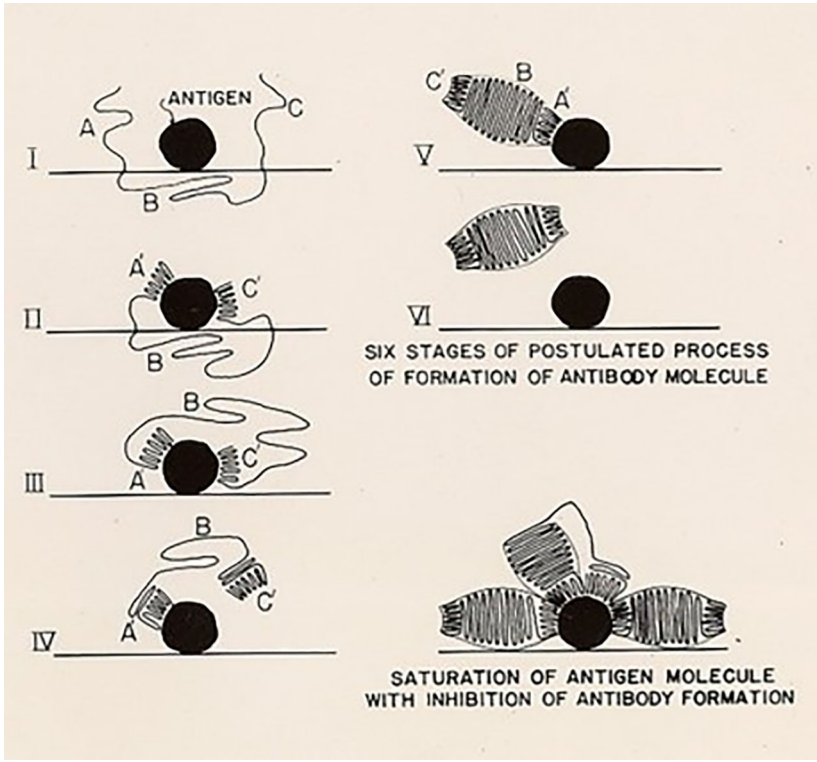

FIG. 1. Six stages in the process of formation of an antibody molecule as the result of interaction of the globulin polypeptide chain with an antigen molecule. B: Central part of the globulin molecule. A and C: Active ends of the globulin molecule that coil up complementarily to the surface of the antigen and thus become specific for this antigen. Lower right: An antigen molecule is surrounded by attached antibody molecules or parts of molecules. *Source:* Linus Pauling, "A Theory of the Structure and Process of Formation of Antibodies," *Journal of the American Chemical Society* 62, no. 10 (1940): 2643–57. Courtesy of the Journal of the American Chemical Society.

justify some speculation as to the nature of the stable configurations of protein molecules."[21]

Pauling speculated that the ends of unspecific globulin molecules were "instructed" by the surface of antigen molecules to assume complementary structures that transformed them into specific antibodies; see Figure 1.

Antigens would serve as templates for the final step of antibody formation: the incompletely folded polypeptide chain of the antibody molecule would

21. Ibid.

reflect the template of the surface structure of the antigen molecule. In contrast to most other template theories, which assumed that the modification of the antibody might involve a re-ordering of the amino acid composition or sequence of unknown mechanism, Pauling regarded such mechanisms as unnecessary.[22]

It has been suggested that in his proposition of complementarity, Pauling was inspired by Emil Fischer's "lock and key" concept of 1894.[23] However, neither in his 1940 paper nor in his later recollections did Pauling ever reference Fischer.[24] Fischer used "lock and key" as a metaphor to describe the high specificity of an enzyme, which he explained by its stereochemical fit to its substrate.[25] However, unlike Pauling, Fischer postulated a rigid structure for enzymes (which he assumed to be proteins) and a selection process with its substrate, concerning which he wrote, "enzymes are as selective as is yeast or other micro-organisms."[26] In this view, enzymes, or what was later called their "active sites," selected those substrates that fitted into their three-dimensional structure, but the substrates did not "instruct" the active sites to form a complementary structure.

2.3. Pauling's Claims of Having Synthesized Artificial Antibodies and the Irreproducibility of His Experiments

In his 1940 paper on antibody formation, Pauling envisioned a far-reaching medical application of his theory, namely the synthesis of artificial antibodies, which, if true, would have revolutionized the production of infectious disease fighting drugs: "An interesting possible method of producing antibodies from serum or globulin solution outside of the animal is suggested by the theory. The globulin would be treated with a denaturing agent or condition sufficiently strong to cause the chain ends to uncoil; after which, this agent or condition would be removed slowly while antigen or hapten is present in the solution in considerable concentration. The chain ends would then coil up to

22. Ibid.

23. Bruno J. Strasser, "A World in One Dimension: Linus Pauling, Francis Crick and the Central Dogma of Molecular Biology," *History and Philosophy of the Life Sciences* (2006): 491–512.

24. Pauling relates the idea of complementary structures of antigen and antibody to Breinl, Haurowitz, Mudd, and Alexander, with some intimation of the same in the early work of Ehrlich and Bordet. See Pauling, "A Theory" (ref. 18); Pauling, "Molecular Basis" (ref. 15).

25. Emil Fischer, "Einfluss der Configuration auf die Wirkung der Enzyme," *Berichte der Deutschen Chemischen Gesellschaft* 27 (1894): 2985–93.

26. Fischer, "Einfluss," translation U. Deichmann.

assume the configurations stable under these conditions, which would be configurations complementary to those of the antigen or hapten." Pauling added that, "many of the experiments suggested above are being undertaken in our Laboratories, with the collaboration of Dr. Dan Campbell."[27]

Because Pauling's work in immunology was considered to have a more direct bearing on the war effort in the 1940s than other projects of basic research, he was permitted by the authorities to continue this research after 1940.[28] He even returned to experimental work, trying to prove that each antibody has only two binding sites, as he had proposed in 1940. But it would be Campbell's alleged success in making artificial antibodies by late 1941, as predicted in Pauling's 1940 paper, which would prove to be the "most exciting news" of Pauling's research at that time.[29]

Details of the method and results of these experiments were published in 1942. Pauling and Campbell used bovine γ-globulin as protein along with several dyes, e.g., methyl blue, as well as pneumococcus polysaccharides as antigens. Heating of protein and antigen to 57° C for several days or weeks would, it was proposed, denature the proteins, i.e., destroy their spatial structure. Under the influence of the antigen, the protein would presumably then form a new spatial structure, complementary to that of the antigen. Treatment with a specific hapten was employed to separate antigen and antibody. The authors claimed that this method was successful and that the experiments resulted in the synthesis of specific artificial antibodies, which is to say that the resulting protein (antibody) solution reacted with the antigen by specifically forming precipitates with it. The authors considered the in vitro production of specific antibodies on an industrial scale possible.[30]

In March 1942, Pauling released a press statement from Caltech announcing the success of his antibody production method that "opened up the possibility of a new method for use in the fight against disease."[31] According to Pauling's biographer Thomas Hager, this announcement in the popular press of

27. Pauling, "A Theory" (ref. 18). A hapten is a small molecule, such as aniline, that stimulates an immune response when attached to a larger one, such as a protein. If different haptens are attached to the same protein, they stimulate different responses.

28. Hager, *The Life* (ref. 8), 261.

29. Ibid.

30. Lily E. Kay, *The Molecular Vision of Life* (New York, Oxford: Oxford University Press, 1993), 177–85; Michel Morange, *A History of Molecular Biology* (London: Cambridge University Press, 1998).

31. Hager, *The Life* (ref. 8), 263.

a discovery before anything had been published, at first worked to Pauling's advantage. Journals such as *Science* and the *Journal of the American Medical Association* noted the work approvingly, and Pauling and Campbell subsequently published their work in these journals.[32] Representatives from pharmaceutical companies offered Pauling financial and technical help.[33]

Despite the warm reception in both the popular press and some of the professional journals, the immunological community remained skeptical. Pauling and Campbell's experiments increasingly met with criticism by colleagues, including those who otherwise strongly appreciated Pauling's work, such as Michael Heidelberger, Karl Landsteiner, and Oswald Avery, as well as by the Rockefeller Foundation, which funded Pauling's protein research. Attempts to reproduce Pauling's results, such as by Landsteiner, remained unsuccessful. Campbell seemed to be the only scientist who could make specific artificial antibodies.[34]

In most cases, colleagues expressed their criticism in private letters as well as expert opinions sent to funding agencies.[35] To mention just a few: Henry B. Bull from Northwestern University wrote in June 1943, "You have my good wishes in your endeavor to prepare artificial antibodies, but I must confess a feeling of pessimism. . . . Frankly, I am not impressed by experimental procedures which work sometimes but which do not at other times, and no cause can be assigned for the failure." Despite being an early promoter of template models, Felix Haurowitz from the University of Istanbul wrote in September 1943, "I tried to repeat your experiments. . . . In such experiments with methyl blue I did not find any trace of antibody. Experiments with resorcinol coupled to diazotized arsanilic acid failed, too." In an expert opinion for the Rockefeller Foundation, microbiologist René Dubos stated that Pauling's views "have received wide notoriety because of his great prestige," adding that many of his colleagues feel "that his claims are based on very insufficient evidence."

In contrast, Elvin A. Kabat, a co-worker of Heidelberger, not only criticized Pauling's method during the latter's visit to the Rockefeller Institute, but also published his criticism: "[the studies] of Pauling and Campbell lack the full

32. Linus Pauling and Dan H. Campbell, "The Manufacture of Antibodies in Vitro," *The Journal of Experimental Medicine* 76, no. 2 (1942): 211–20. Linus Pauling and Dan H. Campbell, "The Production of Antibodies in Vitro," *Science* 95, no. 2469 (1942): 440–41.

33. Hager, *The Life* (ref. 8), 263.

34. Ibid., 265.

35. The quotations in this section (2.3) are from the Linus Pauling Papers, Section LP Correspondences, Oregon State University, Corvallis, Oregon, unless stated otherwise.

details of control experiments necessary for a proper evaluation of their data [such] that the identity of their materials and antibody is far from established." In other words, the studies did not pay attention to unspecific precipitation. For example, dyes similar to the ones they used gave precipitates with normal horse serum. Moreover, they did not exclude the presence of natural antibodies in the starting materials. Kabat also pointed to the fact that Pauling and Campbell's experiment "contains observations which are quite in conflict with the known behavior and properties of antibodies." Therefore he considered Pauling and Campbell's claim that they had been in the "region of antibody-excess . . . extremely unlikely" and "even more unlikely that the precipitate . . . could have been antibody in the usual sense of the term."[36]

Likewise, Jack L. Morrison from the University of Alberta, who had been a postdoctoral fellow with Pauling in 1948 and '49, believed that Pauling and Campbell's method did not demonstrate the production of specific antibodies, but that any specific precipitates of dyes (as antigens) and antibodies would be masked by nonspecific precipitation of other blood proteins by the same dye at the same pH values.[37] Pauling's colleague at Caltech, James Bonner, later commented: "Linus's bad ideas are better than most people's good ones. But it took him a long time to let go of this one though; . . . Dan Campbell came specifically [to Caltech] to work on this matter. They got nowhere."[38]

Pauling's application for a patent on the process of artificial antibody production was rejected by the US Patent Office in May 1943 with the comment that: "The claims [concerning artificial antibodies] are rejected for lack of utility as no evidence has been presented to show that the antibodies alleged to be produced by the method have any utility at all. . . . it would be necessary to carry out further experimentation in order to determine if antibodies are obtained by the method. . . . "

Similarly, the Rockefeller Foundation became critical of Pauling's work on antibodies that they had amply funded. In May 1943, the Foundation wrote to Pauling that "support was given with the hope that the critical central point at issue could be cleared up before the termination of the grant. As we understand it, this critical point is whether antibodies can be manufactured *in vitro* or not.

36. Elvin A. Kabat, "Immunochemistry of the Proteins," *The Journal of Immunology* 47, no. 6 (1943): 513–87.

37. Jack L. Morrison, "The Nonspecific Precipitation of Proteins by Polyhaptenic Dyes," *Nature* 171, no. 4347 (1953): 346–47.

38. Anthony Serafini, *Linus Pauling. A Man and His Science* (London and Sydney: Simon and Schuster, 1989), 101.

From the conferences we have had with our advisers in this field it seems reasonably clear that the question . . . is not unambiguously settled." The Foundation was willing to extend the grant for a year "with the hope that another year of work might result in the publication of experimental evidence in such clear and detailed form that experts could evaluate the results precisely, and that the experiments could be repeated exactly elsewhere." As this statement and the earlier quotes show, repetition of experimental success was a common and expected standard in the evaluation of experiments.

However, Pauling and Campbell did not publish the expected experimental evidence. Pauling also did not admit or explain the failures, but started to belittle the relevance that the antibody research had for him. In his reply to the Rockefeller Foundation, he stated: "Of our work in immunochemistry as a whole . . . the work on artificial antibodies represents only a minor part." The Rockefeller Foundation reduced their support for this work until they finally stopped it in 1944. Pauling abandoned his patent application and stopped working on the production of antibodies, but he never disavowed his experimental papers on specific antibody production; he simply did not mention them anymore. Believing in the correctness of the "instructive" template model, he encouraged his post-doctoral fellow, Frank Dickey, to investigate the production of non-protein adsorbents with specific affinities for predetermined structures, which according to Dickey was successful,[39] but which according to others failed to contribute to significant advances in the field of molecular imprinting (see below) in the next decades.[40] Pauling always seemed to have believed that he had produced specific antibodies albeit very weak ones, but Campbell later blamed one of the laboratory assistants to have shaded the results "to fit what he thought the bosses wanted to see."[41]

Since Pauling did not critically mention his irreproducible experiments later on, it can be assumed that he would not have retracted the papers in which they were published, even if article retraction had been a more common practice at the time. The fact that it was then not common to issue retractions does not, however, mean that the standards of scientific truth and reproducibility were different from today, as can clearly be seen from the

39. Frank H. Dickey, "The Preparation of Specific Adsorbents," *Proceedings of the National Academy of Sciences of the United States of America* 35, no. 5 (1949): 227–29.

40. Xiantao Shen, Changgang Xu, and Lei Ye, "Molecularly Imprinted Polymers for Clean Water: Analysis and Purification," *Industrial & Engineering Chemistry Research* 52, no. 39 (2013): 13890–99.

41. Hager, *The Life* (ref. 8), 265.

contemporaneous statement of the Rockefeller Foundation. The term "scientific truth"[42] was used frequently by philosophers and scientists until the mid-twentieth century, but gradually fell out of use thereafter. The reproducibility of experiments, however, has remained a generally accepted criterion for good science in experimental biology since the early twentieth century.

The relevance of "reproducibility" can also be seen in cases of allegedly ground-breaking biochemical research in Germany in the 1940s that proved irreproducible and for which colleagues published refutations (either immediately or later).[43] In some cases it was expected that the claims would be withdrawn. A young researcher who had obtained widely celebrated results that could not be reproduced by others, subsequently was prevented from receiving a professorship. At the same time, it should be noted that here, too, some renowned scientists were not ready to acknowledge that the research by a young co-worker or a respected colleague was questionable or untenable, and continued to support their colleagues.

2.4. The End of the Era of Direct Template Theories in Immunology

The idea of antibody creation on antigens as direct templates began to be increasingly challenged starting in the early 1940s. Biologists and medical immunologists realized that the immunochemical models were not able to explain basic features of the immunological response, in particular the continuous production of antibodies long after the antigen had disappeared from the organism. According to Thomas Soderqvist, a widespread but unarticulated discontent with template theories was prevalent among immunologists, including some immunochemists such as Colin MacLeod in the early 1950s.

42. "Scientific truth" has remained a contested notion in philosophy of science, and its standards and the methods to achieve it have changed throughout the course of history. See, e.g., Ian Hacking, *Representing and Intervening* (Cambridge: Cambridge University Press, 1983); Lorraine Daston and Peter Galison, *Objectivity* (New York: Zone Books, 2007). However, the notion of truth, not as absolute and unchanging truth, but as reliable knowledge based on experiment and logic, has become—and remains—a fundament of the empirical sciences since the days of Bacon and Galilei in the early seventeenth century, and in modern biology since the end of the nineteenth century. Most empirical scientists hold that experimentally corroborated scientific theories largely reflect aspects of reality. Some early twentieth-century experimentalists in biology regarded the agreement of reproducible observations and/or experiments conducted independently in different fields also as an indicator of conceptual truth (in a probabilistic sense) when scrutinizing a theory.

43. These cases are presented and analyzed in Ute Deichmann, *Flüchten, Mitmachen Vergessen. Chemiker und Biochemiker in der NS-Zeit* (Weinheim: Wiley-VCH, 2001), 329–44.

Indeed, one immunologist in New York proffered that "they knew the instruction theory isn't going to work."[44] But, as Michel Morange made clear, the transition of template models to genome-based molecular selective models "was not linear"; different models of antibody synthesis coexisted after the demise of the direct template theories, and it took many years until "a full molecular description of the mechanism of antibody production" was available and became generally accepted.[45]

Macfarlane Burnet was the first biomedical scientist to attempt to answer the numerous questions that arose from observations of the immune response. His criticism of the template theories did not relate to the question of the chemical basis for the specificity of the antibody combining site, but it questioned the biological basis for the production of the antibodies.[46] Burnet complained that the elegant chemical theories failed to explain the more functional biological aspects of the immune response. According to him, direct template theories paid no attention to the modern knowledge of the importance of enzymes in the mechanisms of intracellular metabolism and synthesis. He also pointed out that these theories, as mentioned before, demanded the probably incorrect assumption of a long-term persistence of antigen throughout the course of antibody formation, and he emphasized that "antibody production is a function not only of the cells originally stimulated, but also of their descendants."[47]

Interestingly, at first, two other template theories were proposed to account for the biological questions. They were, in the wording of Silverstein, indirect template theories (which were shortly thereafter replaced by selective theories): Burnet's notion of 1941 that enzymes in the cell might be adaptively modified so that they would be able to synthesize specific antibodies, which was inspired by the adaptive enzyme hypothesis (see Section 3.3), as well as Burnet and Fenner's theory of 1949 that the genome or RNA would act as an intermediate between antigen and antibody.[48]

44. Thomas Soderqvist, "Darwinian Overtones" (ref. 10).

45. Morange, "The Complex History" (ref. 10).

46. Silverstein, *A History* (ref. 10), 55.

47. Ibid., 55; Warwick Anderson and Ian R. Mackay, "Fashioning the Immunological Self: The Biological Individuality of F. Macfarlane Burnet," *Journal of the History of Biology* 47 (2014): 147–75.

48. Silverstein, *A History* (ref. 10), 54–56; Frank MacFarlane Burnet, *The Production of Antibodies* (New York: Macmillan, 1941); and Frank MacFarlane Burnet and F. Fenner, *The Production of Antibodies*, 2nd ed. (New York: Macmillan, 1949).

The first to turn radically against the established template theories was Niels Kaj Jerne.[49] His proposition of a natural selection theory of the generation of preformed antibodies, in which the antigen plays a selective and amplifying role,[50] to some extent revived Ehrlich's selection theory of spontaneous antibody ("side-chain") generation. In this theory, the blood plasma already possessed preformed antibody molecules with a certain degree of specificity against most foreign substances. Jerne also postulated a stochastic mechanism for the generation of such antibody specificities and a mechanism of selection of the best fitting antibody.[51] His theory was able to explain some of the problems raised by Burnet, as well as the booster phenomenon (the fact that the concentration of antibodies increases dramatically after a second injection of an antigen), where template theories had failed. It also made sense of the phenomenon of avidity—that is, the fact that antibodies produced late in the course of immunization bind better to the antigen than early antibodies.[52]

Jerne's paper received mixed reactions: Haurowitz perceived it only as a revival of Ehrlich's side-chain theory; Pauling rejected it "within five seconds"; and molecular biologists were skeptical, though some supported Jerne.[53] A year later, Jerne's theory was taken up by Burnet and modified into a cellular selection theory.[54] Burnet later recalled that by taking Jerne's Darwinian mechanism as the point of departure and replacing molecules with cell clones and their membrane receptors, "the whole picture fell into shape."[55] Burnet's theory was refined in 1959 by Burnet himself, David W. Talmage, and Joshua Lederberg.[56] According to this refined clonal selection theory, the

49. Soderqvist, "Darwinian Overtones" (ref. 10).

50. Niels K. Jerne, "The Natural-Selection Theory of Antibody Formation," *Proceedings of the National Academy of Sciences in the United States of America* 41 (1955): 849–59.

51. Soderqvist, "Darwinian Overtones" (ref. 10).

52. Ibid.; and Niels K. Jerne, "Antibodies and Learning: Selection versus Instruction," in *Neurosciences*, ed. Gardner C. Quarton, Theodore Melnechuk, Francis O. Schmitt (New York: The Rockefeller University Press, 1967), 200–05.

53. Ibid.

54. Frank MacFarlane Burnet, "A Modification of Jerne's Theory of Antibody Production Using the Concept of Clonal Selection," *Australian Journal of Science* 20, no. 20 (1957): 67–69.

55. Soderqvist, "Darwinian Overtones" (ref. 10). The distinction between "instructive" and selective theories was also used outside the field of biology. Citing Jerne, Noam Chomsky, the founder of generative linguistics, considered this distinction relevant for the correct understanding of the development of language skills; see L. Jenkins, *Biolinguistics. Exploring the Biology of Language* (Cambridge: Cambridge University Press, 2000), 84.

56. Frank MacFarlane Burnet, *The Clonal Selection Theory of Acquired Immunity* (Nashville: Vanderbilt University Press, 1959); David W. Talmage, "Immunological Specificity: Unique

interaction of antigens with pre-existing receptors on lymphoid cells would trigger a signal for cellular differentiation for antibody production, as well as a signal for proliferation to form a clone of daughter cells with identical receptors, capable of identical immunologic responses. Antigens would thus serve to select the appropriate clonal precursor from a much larger population and activate it specifically, thus explaining continued antibody formation, enhanced secondary responses, and changes in the quality of antibodies (by means of selection by the antigens).[57] Lederberg specifically addressed some of the genetic implications of the clonal selection theory, suggesting that immunological specificity is determined by a unique primary amino acid sequence, the information for which is incorporated in a unique sequence of nucleotides in a "gene for globulin synthesis." To account for antibody diversity, Lederberg suggested the existence in precursor cells of a high rate of spontaneous and random mutation of the DNA of the immunoglobulin gene.[58]

The clonal selection theory not only provided reasonable explanations for all of the hitherto inexplicable biological phenomena, but also stimulated new ideas and a vast number of new experiments in immunology and molecular immunology.[59] Even though some immunochemists continued to advocate modified direct template theories (see Section 4.1), the idea that antibody diversity was not a result of an "instruction" through a template, but of genetically determined antibodies and selection by the antigen became generally accepted.[60]

Combinations of Selected Natural Globulins Provide an Alternative to the Classical Concept," *Science* 129, no. 3364 (1959): 1643–48; Joshua Lederberg, "Genes and Antibodies," *Science* 129, no. 3364 (1959): 1649–53.

57. Silverstein, *A History* (ref. 10), 61.

58. Ibid., 60–62.

59. Silverstein, "The Historical Origins" (ref. 11), 17.

60. Silverstein called the era between 1910 and 1960, in which chemists dominated immunology, "the dark ages of immunochemistry," adding that "[w]hile impressive progress was made in this area by Karl Landsteiner and others, it appeared slow and incremental, and brought with it few important generalizations" (456–57). This assessment is reminiscent of that of Marcel Florkin regarding the era of biocolloidy at about the same time. Florkin criticized that "irrelevant theories" related to surface actions, electric charges, and adsorption, as well as the rejection of principles of structural chemistry, retarded the development of scientific biochemistry. Florkin called the period in which biocolloidy strongly influenced biologists' and biochemists' work "the dark age of biocolloidy"; Marcel Florkin, *A History of Biochemistry*, (Amsterdam and London: Elsevier, 1972), 279–80. There is indeed a parallel between the biocolloidy and immunochemistry of the first half of the twentieth century, as both focused on surface action while neglecting biological significance. In addition, at least some of the early representatives of template theories believed in biocolloidy; see, e.g., Ute Deichmann, "'Molecular' versus 'Colloidal': Controversies

3. PHILOSOPHY AND PSYCHOLOGY: THE PRINCIPLE OF PARSIMONY AND POPULAR TEMPLATE THEORIES IN BIOLOGICAL CHEMISTRY

3.1. Pauling's Template Model and the Principle of Parsimony

Neither the various template theories proposed in immunochemistry between 1910 and 1960 nor Ehrlich's selective theory was supported by direct experimental evidence. The widespread preference of template models over selective models was based on other considerations, in particular conceptual and aesthetic predilections and philosophical arguments. For Pauling, the idea of simplicity and a preference for spatial models provided especially strong motivation to propose template models of antibody generation, and to stick by them when they became increasingly questionable.

One reason for the dislike of Ehrlich's theory of preformed antibody formation by later immunochemists was its presumed contradiction to the principle of simplicity and economy, or parsimony, in nature. At the end of the nineteenth century, this principle, known as Ockham's (or Occam's) Razor,[61] was promoted for physics by the physicist and philosopher Ernst Mach in Vienna. Mach advocated for giving preference to simple and economical explanations over more complex ones. In his popular essay "The economical nature of physical enquiry," Mach considered "the simplest and most economical abstract expression of facts" to be the goal of physical science.[62]

in Biology and Biochemistry, 1900–1940," *Bulletin for the History of Chemistry* 32 (2007): 105–18. Nonetheless, starting in the 1930s, key differences prevailed because the major proponents of immunochemistry accepted structural chemistry, a field that was pioneered by Pauling.

61. The principle is attributed to the scholastic scholar William of Ockham, 1287–1347. Ockham refers to a town in England, whereas the Latin spelling "Occam" is due to the fact that the principle was described in Latin literature. Simplicity principles have been proposed in various forms by theologians, philosophers, and scientists, from ancient to modern times. In most cases, the terms "parsimony" and "simplicity" are used interchangeably in the philosophical literature, though a distinction is sometimes made between two distinct senses of simplicity, namely the number and complexity of hypotheses, and the number and complexity of things postulated, the latter often called "parsimony"; see, e.g., Eliott Sober, "What is the Problem of Simplicity?" in *Simplicity, Inference and Modelling: Keeping It Sophisticatedly Simple*, ed. A. Zellner, H. Keuzenkamp, and M. McAleer, M. (Cambridge: Cambridge University Press, 2001), 13–31.

62. Ernst Mach, "The Economical Nature of Physical Enquiry," 1898, Collected lectures dating from 1863 to 1898; first published collectively in English in 1898; see "Ernst Mach," *Stanford Encyclopedia of Philosophy* (2008, rev. 2019), https://plato.stanford.edu/entries/ernst-mach/ (accessed Jul 2021).

Influenced by Mach, Karl Landsteiner, who likewise came from Vienna, introduced this principle to immunochemistry. He criticized Ehrlich's selective theory because it implied the storage of a large amount of different antibodies independent of the presence of antigen: "The number of hypothetical substances postulated makes this conception so uneconomical that the question must be asked whether it is the only one possible."[63] The skepticism regarding Ehrlich's theory grew strongly with the demonstration, in 1906, that protein antigens could be modified chemically to alter their immunological specificity,[64] and that specific antibodies would bind these artificial antigens. This was exploited, in particular by Landsteiner, as a powerful tool to study immunological specificity,[65] and it was also used as an argument against Ehrlich's theory. It would be, it was argued, uneconomical for any organism to store thousands of specific antibodies against substances that could not be found in nature but only in the laboratory.

Pauling combined the simplicity principle with his knowledge in structural chemistry to propose a theory of antibody formation. Since, he argued, at the time, no one had succeeded in setting up a "theory of the structure of antibodies from the observational material,"[66] he developed a theory that was based on the elucidation of "the simplest structure which can be suggested on the basis of the extensive information now available about intramolecular and intermolecular forces, for a molecule with the properties observed for antibodies," and of "the simplest reasonable process of formation of such a molecule."[67] He held that these demands of simplicity would be best fulfilled by a theory, in which the shape of the antibody is "instructed" by that of the antigen, independent of the amino acid composition of sequence: globulin polypeptide chains, possessing "a very great many configurations with nearly the same stability" at the ends assume, under the influence of an antigen molecule, "configurations complementary to surface regions of the antigen."[68]

63. Karl Landsteiner and A. Sturli, "Ueber die Haemagglutine normaler Sera," *Wiener klinische Wochenschrift* 15 (1902): 38–40, C. 19, quoted in Pauline Mazumdar, *Species and Specificity* (Cambridge: Cambridge University Press, 1995), 152.

64. Joshua Lederberg, "Ontogeny of the Clonal Selection Theory of Antibody Formation," *Annals of the New York Academy of Sciences* 546 (1988): 175–82.

65. Silverstein, "The Historical Origins" (ref. 11).

66. Pauling, "A Theory" (ref. 18).

67. Ibid.

68. Ibid. In modern terminology, "configuration" in this context would be "conformation," i.e., a change in the shape but not by cleaving and opening new chemical bonds. Pauling did not provide experimental evidence for this assumption, but cited A. Rothen and Karl Landsteiner

Pauling postulated that "the rule of parsimony," which he defined as "the use of the minimum effort to achieve the result," suggests "that there are only two such [binding] regions" at the two ends of an antibody molecule. However, the bivalence of antibodies had been called into question already for many years, giving rise to the assumption of multivalent antibodies. He even claimed that the two ends of the same antibody were able to adopt different specificities, though such molecules had never been detected (and did not exist). The "rule of parsimony" would answer two questions: "What is the simplest structure which can be suggested" for a molecule with the properties of antibody?; and "What is the simplest reasonable process of formation of such a molecule?"[69]

Pauling assumed that a polypeptide chain of a globulin, built from the same amino acids with the same sequence, could, under the influence of an antigen, form any kind of specific antibody: "The number of configurations accessible to the polypeptide chain is so great as to provide an explanation of the ability of an animal to form antibodies with considerable specificity for an apparently unlimited number of different antigens, without the necessity of invoking also a variation in the amino acid composition or amino-acid order."[70] According to this theory, not only were specific antibodies produced only when needed, but also the complementary structures could be imprinted on any nascent polypeptide chain of globulins; its composition or amino acid sequence was not relevant. Whereas earlier template theories of antibody generation proposed that templates somehow ordered the sequence of amino acids, Pauling considered this approach unnecessarily complex. His idea that polypeptides of any sequence could be "instructed" to fold into a specific shape was simpler.[71]

The idea of simplicity (and intellectual satisfaction) was deeply engrained in Pauling's scientific outlook. This can be seen in his perception of the different approaches to science between himself and Landsteiner: "Landsteiner would ask, 'What do these experimental observations force us to believe about the nature of the world?' and I would ask, 'What is the most simple, general, and intellectually satisfying picture of the world that encompasses these observations and is not incompatible with them?'"[72]

(*Science* 90, no. 85 [1939]) with the vague remark, "the possibility of different ways of folding the same polypeptide chain to obtain different antibodies is worth considering."

69. Pauling, "A Theory" (ref. 18).

70. Ibid.

71. Hager, *The Life* (ref. 8), 238.

72. Ibid., 240.

The ideas of parsimony and simplicity had been important principles in the history of chemistry. As Alan Rocke has shown, "simplicity" played an important role in early nineteenth-century chemistry, where atomists used simplicity assumptions in addition to analytical data in their work on atomic weight and molecular formulas.[73] Roald Hoffmann *et al.* perceive an affinity of the simplicity or parsimony principle, which they relate to "Ockham's Razor," and intuition in chemistry.[74] Referring to Bertrand Russell, the authors assert that "the utility of Ockham's Razor in the selection and classification of reaction mechanisms has proven itself in chemistry, just as it has in various other areas of natural science," and that "Ockham's Razor must indubitably be counted among the tried and useful principles of thinking about the facts."[75] Their relating simplicity and parsimony to intuition in many respects fits the way Pauling used simplicity, both for better and for worse: Intuition "figures prominently in the strong pull on us toward the simple, the logical, and the beautiful . . . 'Intuitive' is, probably, the best characterization of the law of parsimony, Ockham's Razor. It is also intuition that sometimes leads to the oh so many blind alleys, if not mistakes, of our sciences."[76]

Mary Jo Nye thinks that for Pauling, "molecular shape and molecular architecture were becoming central to thinking about mechanisms of chemical reactions in biological processes."[77] Similarly, Alberto Cambrosio *et al.* have argued that Pauling's predilection for three-dimensional shapes found its expression also in his detailed images, and in particular in his 1940 paper (see Figure 1), which these authors construed not only as illustrations, but also as visual arguments that went beyond Pauling's analyses.[78] Interestingly, Nye perceives in Pauling's "persistent advocacy of the valence-bond theory over the molecular orbital theory an instance of his preference for what might be

73. Alan Rocke, "Chemical Atomism and the Evolution of Chemical Theory in the Nineteenth Century," in *Tools and Modes of Representation in the Laboratory Sciences*, ed. Ursula Klein (Berlin: Springer, 2001) 1–11.

74. Roald Hoffmann, Vladimir I. Minkin, and Barry K. Carpenter, "Ockham's Razor and Chemistry," *HYLE—International Journal for Philosophy of Chemistry* 3 (1997): 3–28.

75. Ibid.

76. Ibid.

77. Mary Jo Nye, "Paper Tools and Molecular Architecture in the Chemistry of Linus Pauling," in *Tools and Modes of Representation in the Laboratory Sciences*, ed. Ursula Klein (Berlin: Springer, 2001), 117–32.

78. Alberto Cambrosio, Daniel Jacobi, and Peter Keating, "Arguing with Images: Pauling's Theory of Antibody Formation," *Representations* 89, no. 1 (2005): 662–99.

called chemical intuition and the chemist's way of seeing things."[79] The valence bond theory, to which Pauling had contributed, assumes that electrons in a molecule are simply the electrons in the original atomic orbitals, with some used while bonding. In other words, the valence bond theory treats electrons as if they are "localized" on the atoms themselves. In contrast, the molecular orbital theory, which by and large has replaced the valence bond theory, except in teaching, rejects the idea of molecules as aggregates of distinct atoms, and claims that electrons are "delocalized" in the molecule. According to Nye, "structural chemists in Pauling's view thought of molecules atom by atom, and attributed shapes and visual images to molecules that are compositions of atoms in contrast to chemical physicists' treatment of the molecule as a whole unit."[80]

Francis Crick, who turned from physics to molecular biology—he co-discovered with James Watson the double helix structure of DNA—pointed to the pitfalls of using the principle of parsimony in biology, which, he argued, have been due to the fact that biological systems are results of evolution. He held that whereas "Occam's razor is a useful tool in the physical sciences, it can be a very dangerous implement in biology. It is thus very rash to use simplicity and elegance as a guide in biological research." He advised biologists to constantly keep in mind that "what they see was not designed, but rather evolved." According to him, "elegance and simplicity are, in biology, dangerous guides to the correct answer," and the only constraints that can be used as a "guide through the jungle of possible theories" are experimental tests.[81]

The generation of antibodies as an evolved property of the immune system is an example, where simplicity and elegance failed as guiding principles. The "instructive" template models according to which antibodies were generated in a direct way, "instructed" by the surface shape of the antigen, were theoretically appealing, because they were regarded as simpler than selective models. However, as in many other cases in biology (see Section 3.3), the immune system does not work that way in nature but generates antibodies in a more complicated process, through which they are produced randomly and subsequently selected by the antigen.

79. Mary Jo Nye in letter to author, 7 Aug 2020.

80. Ibid.

81. Francis Crick, *What Mad Pursuit: A Personal View of Scientific Discovery* (New York: Basic Books, 1988), 138.

3.2. Simplicity Arguments Regarding Protein Structure

Both the potential fruitfulness and the pitfalls of the principle of simplicity are also clearly evident in early research on protein structure. Protein biochemist and historian of biochemistry Joseph Fruton pointed to the fact that the simplicity required by Ockham's Razor is different from the simplicity sought through geometric or other mathematical representations.[82] He observed that despite the fruitfulness of the search for simplicity through geometrical or numeric representations of natural objects and processes as part of the methodology of the chemical and biochemical sciences, many of the ideas that were once hailed because "they were considered to be simple and elegant descriptions of natural objects" were later discarded.[83] Fruton, similar to Roald Hoffmann, relates a preference for simplicity to intuition, deploring, at the same time, the fact that on many occasions, "in striving for simplicity and elegance, chemists and biochemists have been led astray by their penchant for numerology because they allowed imagination and intuition to take precedence over attention to the limits of their experimental procedures and the validity of their empirical data."[84]

Among the examples of protein chemists in the 1920s and '30s having been led astray by arguments of simplicity are those who favored periodicity hypotheses of protein structure. Some protein chemists believed they had found numerical regularities in the amino acid composition and sequence of protein structure.[85] The existence of sequence regularities was asserted, for example, by the well-known protein chemists Theodor Svedberg and Max Bergmann. Bergmann and Carl Niemann's 1937 hypothesis of numerical regularity in protein structures became popular for several years, though Bergmann himself had already abandoned this hypothesis by 1938. A numerical regularity of amino acids in functionally important proteins would clearly have marginalized the importance of amino acid sequences for protein function.

82. Joseph S. Fruton, *A Skeptical Biochemist* (Cambridge, MA: Harvard University Press, 1992), 46–47.

83. Ibid., 47.

84. Ibid., 48.

85. Some fibrous proteins, such as silk and collagen possess some kind of regularities in their amino acid sequences. Thus, glycine is found in almost every third position of the polypeptide chain of collagen. However, the amino acids in antibodies or in proteins that are involved in regulatory processes in the cell or in the body, and in particular in enzymes and hormones, do not have a regular sequence.

In a paper with Niemann in 1939, Pauling considered amino acid periodicity highly probable: "Although there is no direct and unambiguous experimental evidence confirming the idea that the constituent amino acid residues are arranged in a periodic manner along the peptide chain, there is also no experimental evidence which would deny such a possibility, and it seems probable that steric factors might well cause every second or third residue in a chain to be a glycine residue, for example."[86] It can be assumed that Pauling's advocacy of periodicity contributed to his conviction of the irrelevance of sequence for protein structure and function.

The concept of "aperiodicity" related to biological specificity and the ability to carry information in biological systems was introduced not by a chemist, but by a physicist, namely Erwin Schrödinger, in his 1944 book *What is Life?*, where he compared a gene to an "aperiodic crystal" that contained the code for life processes.[87] A few years later, after their discovery of the DNA double-helix structure, Watson and Crick emphasized the *irregularity* of the order of bases in one DNA strand as the basis for a genetic code based on base sequences.[88] Pauling does not seem to have attributed any importance to the relevance of "aperiodicity" for biological specificity, nor any importance to the discourse regarding information in the 1940s and '50s, for in his opinion, genetic information was conveyed by three-dimensional templates.

3.3. The Misleading Comparison of "Selection" and "Template" Theories

The fact that many chemically oriented immunologists proposed and preferred template models, whereas biologically oriented immunologists supported selection models, seems to suggest that the preference for either template or selection models is discipline-specific. This comparison overlooks, however, that "template" and "selection" are not equivalent categories; templates are not the opposite of selection.

The biologists did not suggest a different chemical mechanism for the recognition of antibody and antigen than the chemists; they simply dealt with different aspects of the immune response. Chemists seemed oblivious to the fact that they were dealing not only with the interaction between two types of

86. Linus Pauling and Carl Niemann, "The Structure of Proteins," *Journal of the American Chemical Society* 61, no. 7 (1939): 1860–67.

87. Erwin Schrödinger, *What is Life?* (Cambridge: Cambridge University Press, 1944).

88. James D. Watson and Francis H.C. Crick, "Genetical Implications of the Structure of Deoxyribonucleic Acid," *Nature* 171, no. 4361 (1953): 964–67.

molecules, but also with a biological process that was a result of evolution—antibodies were species-specific proteins. That the preferences for the different models are not (only) discipline-specific can also be seen from the fact that not only biologists, but also many of the renowned immunochemists rejected Pauling's template theory, and that some biologists suggested (indirect) template theories.

The notion of template in Pauling's and others' theories has a double meaning. On the one hand, it means the complementary interaction between surface regions of antibody and antigen, while on the other, it also means the generation of specific antibody shapes by way of "instruction" of a three-dimensional template. The assumption of complementary interactions between parts of the molecules was not controversial at all, even among biologists, whereas the assumption of the synthesis of antibodies onto templates, for the reasons outlined above, was increasingly disputed and ultimately rejected.

But selection by itself is not an alternative for template, because "selection" does not specify a mechanism of antibody creation, it only relates to the selection of pre-existing ones. From this perspective, Pauling's statement that the selection theory "wasn't a theory at all since it didn't specify anything about the molecular mechanism"[89] has a certain degree of legitimacy. Only with the later molecular biological explanation that antibodies are genetically coded for and differ in their amino acid sequences did selection conceptually replace direct template theories. The importance of weak chemical forces, such as hydrogen bonds, and complementarity, propagated so strongly by Pauling, nevertheless always remained undisputed, also by biologists.

Complementarity and template models that were based on sequences of bases or amino acids are of great importance in biology, and in particular with regard to DNA replication, the genetic code, and protein biosynthesis, as Horace Freeland Judson has highlighted in his review of early molecular biology.[90] "Complementary replication, and the closely related concept that molecules can have surfaces that act as templates on which other molecules are formed, permeate biology today with immense explanatory power."[91] For example, DNA replication occurs when the two strands unwind, i.e., *the*

89. Gunther Stent to Niels K. Jerne, 27 Feb 1956, cited in Soderqvist, *Science as Autobiography* (ref. 10), 200.

90. Judson, *The Eighth Day* (ref. 3), passim; see also Watson and Crick, "Genetical Implications" (ref. 88).

91. Judson, *The Eighth Day* (ref. 3), 117.

three-dimensional structure is dissolved, and each becomes the template on which a new strand is formed, "the nucleotides in the growing strand matching themselves one by one to the base sequence on the parent strand."[92] Here, the dissolution of the three-dimensional DNA structure is the pre-requisite for the molecule to act as a template.

As with the theories of antibody synthesis, the first theories of the genetic code involved direct templates. George Gamow's 1954 DNA code was a direct template code based on a stereochemical fit of DNA base sequences and amino acids, in which the four DNA bases directly coded for twenty amino acids, with an overlapping triplet code that acted as a physical template.[93] In part stimulated by, but deviating from, Gamow's hypothesis, Francis Crick proposed, a few years later, the sequence hypothesis of protein synthesis, in which the DNA base sequence only specified the amino acid sequence of a protein without forming a spatial template.[94] Similarly, three-dimensional template models were prevalent also in theories of enzyme synthesis, where it was believed that the active site of an enzyme was directly molded on the inducing substrate. This view was also abandoned in the mid-1950s, when increasing evidence supported the idea of a genetic determination of enzyme specificity, with the inducing substrate regulating the quantity of enzyme-protein synthesis.[95] Subsequently, adaptive enzymes were shown by Jacob and Monod to be inducible enzymes arising through the selection of pre-existing genes.[96]

As these examples show, many of the processes that involve templates resemble "instructive" processes, although, as Niels Jerne has made clear, they are in fact based on selective mechanisms.[97] Thus, in the template models of DNA replication or protein biosynthesis, the "template" molecules do not "instruct" other polynucleotides to assume a specific shape, but they in fact select the nucleotides of DNA or transfer RNA through complementary interactions, which are based on sequences, not three-dimensional shapes.

92. Ibid. 145.

93. George Gamow, "Possible Relation Between Deoxyribonucleic Acid and Protein Structures," *Nature* 173 (1954): 318.

94. Francis H.C. Crick, "On Protein Synthesis," *Symposia of the Society for Experimental Biology* 12 (1958): 138–63. For a more detailed description of direct template models in biological chemistry, see Strasser, "A World" (ref. 23).

95. Joshua Lederberg, "Comments on Gene-Enzyme Relationship," in *Enzymes: Units of Biological Structure and Function*, ed. Oliver H. Gaebler (New York: Academic Press, 1956), 161–74.

96. Morange, "The Complex History" (ref. 10); Jerne, "Antibodies and Learning" (ref. 52).

97. Jerne, "Antibodies and Learning" (ref. 52).

Jerne has pointed out that the generation of antibodies is one of several cases in the history of biology in which an "instructive" theory was initially proposed to account for the underlying mechanism, but was later replaced by a "selective" theory: "Looking back into the history of biology, it appears that wherever a phenomenon resembles learning, an instructive theory was first proposed to account for the underlying mechanisms. In every case, this was later replaced by a selection theory."[98] He asserts that in this process, the logical arguments for the idea of selection have been enforced by experimental evidence. He made it clear that "selection refers to a mechanism in which the product under consideration is already present in the system prior to the arrival of the signal, and is thus recognized and amplified."

Jerne's assessment that many biological processes that were at first perceived as a result of an "instruction" by direct templates were in fact based on pre-existing entities, such as nucleotides and specific antibodies, and a selection mechanism, shows that the preference for template theories in immunochemistry was not an isolated case in the history of biomedical research. The fact that "instructive" template models were aesthetically pleasing and simpler than alternative models based on the random generation of specific proteins and subsequent selection by ligands may help explain their widespread use in immunology and other areas of biology. All these cases highlight the crucial role that experimental evidence has had for the corroboration of the "logical arguments of the idea of selection."

4. RECEPTION OF PAULING'S ANTIBODY RESEARCH, 1940-2018

4.1. Overview of the Reception

A citation analysis, with the help of the Web of Knowledge engine in all databases of the BIOSIS Citation Index, shows that Pauling's 1940 paper on antibody formation was cited altogether a total of 776 times between 1940 and 2019. Interestingly, the paper did not receive the majority of citations in the first years after publication, but was actually cited far more often starting in the 1960s, and then especially after 2000. Between 2008 and 2018, it received up to 30 citations per year; see Figure 2.

98. Ibid. Among Jerne's examples are the selection of (pre-existing) antibiotic-resistant bacteria by antibiotics, and the selection by messenger RNA of certain species of amino acid-charged transfer RNA molecules in protein biosynthesis.

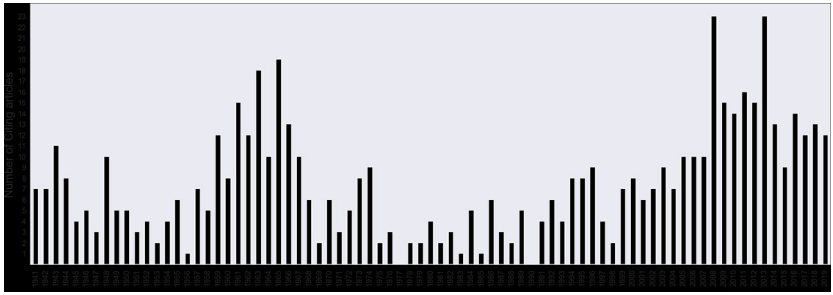

FIG. 2. Number of articles per year that cited Pauling's 1940 paper, 1941–2019.
Source: Web of Knowledge engine in all databases of BIOSIS Citation Index.

During the first ten years after its publication, the paper was cited—both approvingly and critically—predominantly by immunochemists, with the number of citations decreasing in the 1950s, when increasing evidence began to call template theories of antibody formation into question. The 1960s saw a new rise in the number of citations of the paper, reflecting a new interest in immunology that was probably caused by the revelation of the chemical structure of antibodies (by Gerald Edelman and Rodney Porter in 1959) and the awarding of the Nobel Prize to the two immunologists Macfarlane Burnet and Peter Medawar (for the discovery of acquired immunological tolerance, in 1960). Though outdated for many researchers, direct template theories retained their attraction for others.[99] Early on, Pauling's paper was also cited by chemists pursuing molecular imprinting of polymers, a chemical technology that became very popular starting in the 1990s (see Section 4.3).

In the following, I briefly examine the early interest in Pauling's method of antibody production in Germany during World War II. I then scrutinize the citations Pauling's antibody work received in molecular imprinting, and afterward deal with articles that cite other aspects of Pauling's paper.

4.2. Uncritical Appreciation in Germany during the Second World War

Interestingly, extensive research on artificial antibody production inspired by Pauling's work took place in Nazi Germany during the Second World War.

99. An example is immunochemist Haurowitz who, in 1965, without much influence, tried to integrate the template concept, as proposed by himself (and Breinl) in 1930 and Pauling in 1940, into new developments in molecular biology such as the genetic code, and to address the criticism raised by biologists against immunochemical models of antibody generation; Felix Haurowitz, "Antibody Formation and the Coding Problem," *Nature* 205 (1965): 847–51.

The center of this research was the Kaiser Wilhelm Institute for Biochemistry in Berlin under renowned biochemist Adolf Butenandt. Citing Pauling's supposedly successful artificial antibody production, Butenandt emphasized in his report to funding authorities the extraordinarily great medical, military, and economic importance of this research, because artificially produced antibodies would enable novel specific treatment of infectious diseases and epidemics.[100] The Reich Research Council classified artificial antibody research as highly relevant to the war effort, and several of Butenandt's coworkers were exempted from military service in order to conduct research along these lines.[101] Although it is not surprising that Butenandt and the German military and research authorities were interested in this research, it is surprising that all of the available research reports claim that the method was successful and antibodies had been produced.[102] The research projects were discontinued at the end of the war. Afterward, two critical articles appeared in German journals: In 1946, Hans Friedrich-Freksa reviewed Pauling's works on antibody synthesis in detail, concluding that "because of their importance, these experiments need further confirmation."[103] Ulrich Westphal, who had reported positive results during the war, cautioned against uncritical acceptance of Pauling's results on the grounds that they had received surprisingly little confirmation and were repudiated by many researchers.[104]

100. Butenandt, report to Reich Research Council, 7 Jan 1944, archives of the Max Planck Society, Abt. III, Rep. 84/1, Nr. 593.

101. Ute Deichmann, "Proteinforschung an Kaiser-Wilhelm-Instituten 1930–1950 im internationalen Vergleich," *Ergebnisse* 21 (2004), Präsidentenkommission "Geschichte der Kaiser-Wilhelm-Gesellschaft im Nationalsozialismus" der Max-Planck-Gesellschaft, Berlin 2004. As stated earlier, Pauling's project of artificial antibody synthesis was discontinued during the war because he could not provide reliable evidence for it.

102. Reasons for the fact that the reports contained only positive results have been discussed in Deichmann, "Proteinforschung" (ref. 101). Among them were the high value attributed to authority and the discouragement of criticism, as well as the lack of professional competence in protein research due to the expulsion of Jewish biochemists. We can also assume that some of the young German researchers were interested in maintaining their exemption from military service by reporting promising results. None of these allegedly successful results was ever published.

103. Hans Friedrich-Freksa, "Arbeiten von L. Pauling und Mitarbeitern über die Bildung von Antikörpern in vitro und über Haptene mit 2 und mehr Haftgruppen," *Zeitschrift für Naturforschung* 1 (1946): 44–46.

104. Ulrich Westphal, "Zur Frage der Antikörperbildung außerhalb des tierischen Organismus," *Zeitschrift für Naturforschung B* 4, no. 1 (1949): 53–54.

4.3. Appreciation of Pauling as Pioneer of Molecular Imprinting

The sharp rise in citing papers in the field of Molecularly Imprinted Polymers (MIPs) led to a strong increase, starting in 1990, in the citation of Pauling's 1940 paper. The idea of MIP is to obtain specific polymers using the molecular imprinting technique in which the polymer forms around a template molecule that binds either with weak bonds or with covalent bonds that can be easily broken. Once the template is separated from the polymer, the latter should have a cavity with the same shape as the template. Pauling's 1940 paper was cited 75 times between 2014 and 2019, with the majority of the citing papers (63%) coming from the field of MIP. Of the 53 known funding agencies, most were from the US (51%) and China (26%).

According to Hakan S. Anderson and Ian A. Nicholls, while preliminary papers on molecular imprinting (MI) appeared in the 1930s by the Soviet chemist M. V. Polyakov, modern MI was inspired by Pauling and invented by Dickey in the late 1940s.[105] In the 1970s, Günter Wulff, coming from organic and macromolecular chemistry, introduced MI in synthetic organic polymers. Another widely cited author in the field is Klaus Mosbach, who like Wulff was not inspired by Pauling himself, but acknowledged that some of the first attempts in this field were inspired by Pauling's hypothesis of antibody formation.[106] The number of original publications in molecular imprinting increased from one in 1931 to ten in 1990. It then started to rise exponentially, increasing to 80 in 1997[107] and then to 914 in 2011, with a remarkable increase in the percentage of what were termed "interesting Chinese papers."[108]

Interestingly, it was the prospect of the artificial synthesis of antibodies or enzyme-like polymers that seemed to have been a major impetus for this research. Praising the contributions by Pauling and Dickey, Anderson and Nicholls claim that "mankind has for decades benefited from the *in vitro* use

105. Hakan S. Anderson and Ian A. Nicholls, "A Historical Perspective of the Development of Molecular Imprinting," in *Molecularly Imprinted Polymers: Man-made Mimics of Antibodies and Their Application in Analytical Chemistry*, ed. Boerje Sellergren (Amsterdam, Lausanne, and New York: Elsevier, 2000), 1–21.

106. Klaus Mosbach and Olof Ramström, "The Emerging Technique of Molecular Imprinting and Its Future Impact on Biotechnology," *Nature Biotechnology* 14 (1996): 163–70. This review paper stands out with its high number of self-citations: 48 self-citations out of 88 total references (54.5%).

107. Anderson and Nicholls, "A Historical Perspective" (ref. 105).

108. Günter Wulff, "Fourty Years of Molecular Imprinting in Synthetic Polymers: Origin, Features and Perspectives," *Microchimica Acta* 180, no. 15–16 (2013): 1359–70.

of antibodies." They considered the validity of Pauling's and Campbell's experimental papers on artificial antibody synthesis irrelevant ("It appears pointless to discuss the validity of these findings today.") and highlighted the similarity of Pauling's work to today's molecular imprinting: "It is noteworthy that the procedure was in essence similar to what today is called bio-imprinting."[109] In both cases the procedures were based on "instructive" models and used direct template methods.

This statement gives rise to the question of how it was possible that Pauling's paper, which proposed a mistaken hypothesis and an experimental method that did not work, could be cited as a paper that stimulated a methodology along similar lines, without prior analysis of the failure and suggestions for improvements. It is true that mistaken theories (I do not distinguish among theory, hypothesis, or model) in principle can give rise to fruitful research. An example is the 1964 Special Virus Leukemia Program of the National Cancer Institute, which predicted leukemia viruses before a human cancer virus was known to exist, but ultimately failed to find any such viruses.[110] According to Robin Scheffler, the program, despite this failure, among other things laid the groundwork for the identification of HIV. This program was based on epidemiological observations; it could also be related to the discovery of cancer viruses in animals as early as the beginning of the twentieth century, when Peyton Rous discovered them in chickens. In addition, the program took place at a time when evidence of the existence of human cancer viruses was increasing.[111] And it should be emphasized that unlike Pauling's predictions of experimental practices, the theory of human cancer viruses was never experimentally refuted.

This example shows that it is important to distinguish between theories that are proposed to explain existing phenomena or experimental results, and those that provide instructions for practices that did not exist prior to the theory. In the former case, mistaken theories can give rise to further experiments and new testable theories. In the latter case, the theory, at least the part that predicts new experimental practices, is not fruitful once it has been refuted by experiment.

109. Anderson and Nicholls, "A Historical Perspective" (ref. 105), 3 and 5.

110. Robin Wolfe Scheffler, *A Contagious Cause. The American Hunt for Cancer Viruses and the Rise of Molecular Medicine* (Chicago: University of Chicago Press, 2019).

111. In the 1960s, Werner, Gertrude Henle, and Harald zur Hausen showed for the first time that a virus (the Epstein-Barr virus) can transform lymphocytes into cancer cells. In 2008, zur Hausen shared a Nobel Prize for his work on cancer viruses.

This reasoning can also be applied to Pauling's 1940 theory. It contained parts that were fruitful, in particular his highlighting of the (already known) immensely important principle of complementarity and of the role of weak forces in macromolecular interaction. However, his claim that the existence of direct "instruction" templates predicted by the theory could be successfully applied experimentally to produce artificial antibodies, did not result in successful research: the experimental method ended up failing. Two subsequent publications purporting its success claimed results that were irreproducible, and the attempt by Pauling's postdoctoral fellow Dickey to use Pauling's method for molecularly imprinting non-protein molecules had questionable results. Therefore, the claim of researchers of molecularly imprinted antibodies, other proteins, and non-protein molecules that their procedures, which they assert to be essentially similar to Pauling's, had been successful (a contention that is supported by the strong rise of papers in this field since the 1990s), deserves further examination.

It should be noted here that this article does not attempt to give a comprehensive assessment of the expansive research field of molecularly imprinted polymers, a field that has diverse subfields, the success of which seems to be very different. Instead, the article points to some doubts, raised by practitioners themselves after decades of research, about the claims of the field's far-reaching success. These doubts particularly relate to the MI of large molecules, such as artificial antibodies, and large-scale industrial applications. One of the pioneers of MI, Günter Wulff, offered this pithy assessment in 2013, forty years after the begin of MI research in synthetic polymers: "Imprinting of high-molecular-weight biopolymers is still problematic."[112] That same year, Jenna Bowen *et al.* reached a similar assessment: "Today, the perception of molecular imprinting by the wider research community remains mixed whilst its commercial impact is modest. . . . The strength of molecular imprinting lies in its ability to deliver synthetic receptors capable of targeting low molecular weight molecules in non-polar environments. What they are not good at is being solubilised, labelled or linked to enzyme reporter systems."[113]

The gap between far-reaching promises and actual achievements is most apparent regarding industrial applications. A review article on applications of

112. Wulff, "Fourty Years" (ref. 108).

113. Jenna L. Bowen, Panagiotis Manesiotis, and Chris J. Allende, "Twenty Years Since 'Antibody Mimics' by Molecular Imprinting Were First Proposed: A Critical Perspective," *Molecular Imprinting* 1 (2013): 35–40.

molecularly imprinted polymers, focusing on the development of sensors for targets, described there being a large number of reported successes, yet the authors conclude that "commercialization of MIPs has been a slow process."[114] Though they consider it "reasonable to speculate that commercial applications of MIPs to sensors will appear in the near term," pointing, for instance, to their potential for the analysis of organic compounds in wine, the authors nonetheless had to concede that most commercial applications involved materials for separation rather than the MIPs themselves. The authors mention as one of the greatest impediments to the development of MIP-based materials for sensors the "occurrence of nonspecific binding to the templated material," which is reminiscent of similar problems with Pauling and Campbell's method.

According to Gabriel Lemcoff and Steven Zimmerman, the enormous potential for MIPs, be it in chemosensor applications or as antibody replacements in bioanalytical assays, to a large extent has not been realized because of their inherent limitations, among which are issues such as the heterogeneity in binding affinities, slow mass transfer in and out of the polymer matrix, lack of specificity, and overall low binding affinity.[115] The failure is also demonstrated by the fact that, even today, there are no commercial molecularly imprinted polymers that function as drugs, sensors, or catalysts, even though there are many claims in this direction and an impressive number of scientific publications. Lemcoff asserts, "Today we are flooded by papers on MIPs; it is a fashionable field. Papers are cited quite well, but I have my doubts that most of the claims really work. People still call it even today a 'most promising approach', but after 40 years in which nothing came out of this method, one should start doubting whether it works. If it had been useful, it would have become commercial. But I have not seen any of this."[116]

The idea that successful industrial application can serve as a measure of scientific success, is reminiscent of the "engineering ideal" of scientific practice around the turn of the twentieth century, according to which predictive manipulation of entities was a means of establishing scientific truth.

114. Joseph J. BelBruno, "Molecularly Imprinted Polymers," *Chemical Reviews* 119 (2019): 94–119.

115. Steven C. Zimmerman and N. Gabriel Lemcoff, "Synthetic Hosts via Molecular Imprinting—Are Universal Synthetic Antibodies Realistically Possible?" *Chemical Communications* 1 (2004): 5–14.

116. G. Lemcoff, conversation with author, Beer Sheva, 11 Apr 2019.

4.4. Citation of Pauling's Paper as Origin of the Idea of Multiple Conformation of Proteins

There have been a number of researchers who have cited Pauling's paper as pioneering the idea that proteins can exist in multiple conformations, such as Dan Tawfik, who cites Pauling's 1940 paper as a forerunner to the idea that antibodies, or proteins in general, have the ability to exist in many conformations with nearly the same stability, out of which functional ones are selected.[117] Like other researchers, Tawfik here seems to have overlooked that (i) Pauling did not demonstrate the existence of proteins in many conformations with nearly the same stability, but followed an earlier unproven assumption of Landsteiner to this effect, and (ii) that Pauling did *not* envisage a *selection* of the functional conformations, but claimed that they were *instructed* by the ligands (antigens). Tawfik, in contrast to Pauling, does not call into question the fact that antibody diversity is based on changes in DNA sequences of the coding genes. He rejects as completely misleading Pauling's idea, expressed *inter alia* in Figure 1 of his (Pauling's) paper (see Section 2.1), that antibodies are folded on antigens as templates. The conformational diversity of the antibodies Tawfik is studying is not a response to a ligand or template, but is generated in the germline, and gives way to the generation of more specific antibodies with antibody maturation.[118]

Like Tawfik, Peter Schultz and Richard Lerner also consider Pauling (and Haurowitz) to be the originators of the idea of multiple conformations of proteins, in this case antibodies, an assessment that can be inferred from their statement that "the early hypothesis of Felix Haurowitz and Pauling that germline antibody-combining sites can adapt to many shapes . . . seems now to be correct." [119] Well aware of the roles of mutations and sequence diversity in antibody diversity, Schultz and Lerner here disregard the fact that Pauling's "instructive" model of antibody "adaptation" requires ligands as "instructors" for the different conformations. Several other researchers of protein diversity have likewise cited Pauling's paper without paying attention to the basic

117. Dan S. Tawfik, "A Personal Reflection on the Chemistry-Biology Interface," *Israel Journal of Chemistry* 59, no. 1–2 (2019): 23–28; see also Leo C. James and Dan S. Tawfik, "Conformational Diversity and Protein Evolution—A 60-Year-Old Hypothesis Revised," *Trends in Biochemical Sciences* 28, no. 7 (2003): 361–68; and Leo C. James, Pietro Roversi, and Dan S. Tawfik, "Antibody Multispecificity Mediated by Conformational Diversity," *Science* 299 (2003): 1362–67.

118. Tawfik, conversation with author, Rehovot, 22 May 2019.

119. Peter G. Schultz and Richard A. Lerner, "Completing the Circle," *Nature* 418 (2002): 485.

difference between Pauling's "instruction" template model and their own models, which include mutation and selection.

4.5. Citation of Pauling's Paper in the Context of Intrinsically Disordered Proteins

Another area where misplaced approbation for Pauling's contributions can be found, is in the field of intrinsically disordered proteins (IDPs). Some papers cite Pauling as having pioneered this notion of IDPs because of the emphasis he placed on multiple conformations of proteins independent of their amino acid sequence. To this day, the question of how proteins fold is still not well understood. However, recent research has actually confirmed the relevance of the amino acid sequence for function even for disordered proteins, thereby shedding doubt on the universality of Pauling's concept that complementarity is in all cases dependent on proteins' three-dimensional shape. And while it is true that the phenomenon of dysfunctional disordered proteins, such as found in Alzheimer's disease, does seem to confirm the central role of protein folding for function, the phenomenon of functional IDPs or intrinsically disordered regions (IDRs) within otherwise folded proteins reveals a different basis for function than mere molecular structure.

Such IDPs have few hydrophobic residues but many hydrophilic ones. Totally disordered proteins are rare, but partially disordered proteins are abundant and they are functional. They frequently occur, for example, in signal transduction, because they tend to release the ligand more easily than completely folded proteins. They can undergo specific interactions with folded proteins by using dedicated recognition motifs that are encoded in the primary sequence of the IDP (or IDR). In several of these unstructured recognition motifs, the binding propensity can be modulated by post-translational modifications, adding another layer of regulation to this recognition.[120] This means that in the functional IDPs, the ligand does not induce a particular shape as demanded by Pauling's template theory, and therefore citing Pauling for having contributed to this field is misleading given his singular reliance on the three-dimensional template. Instead, a specific primary structure, i.e., amino acid sequence, is the pre-requisite for binding. Thus, the existence of functional IDPs and IDRs calls into question the theory that protein shape is the

120. I thank Kay Hofmann for the explanation of the mode of action of IDPs (conversation with author, 23 Jul 2019).

sole requirement for their specificity. Instead they confirm the central role of sequence in establishing functionality.

5. SUMMARY AND CONCLUDING REMARKS

5.1. Simplicity, parsimony, and periodicity versus complexity and aperiodicity

Pauling's inclination for the philosophical ideas of simplicity and parsimony, together with his belief in a three-dimensional notion of complementarity, played a major role in leading him to propose an immunochemical "instruction" template theory of antibody generation. He and others who suggested similar models claimed a direct molding of antibody shapes onto that of antigens, i.e., the antigen "instructs" the antibody's folding. The underlying working assumption was that direct template theories would be simpler and more "economical" than the earlier theory of antibody generation as put forth by Paul Ehrlich, and according to which a huge amount of randomly generated different antibodies was preformed and stored in the organism until they were selected and stimulated by specific antigens.

The early immunochemical models by Pauling and others explained the molecular interaction between antigen and antibody. But they could not account for the complexity of the actual immune responses, and ultimately these template models were replaced by theories according to which the entities in question were genetically preformed and selected by outside agents such as antigens (so-called selective theories). Pauling's wide knowledge and strong belief in the correctness of his own theories had been instrumental in vigorously promoting novel concepts such as molecular specificity and molecular disease.[121] But such a belief as well as the general lack of self-criticism that his own students and colleagues attributed to him, prevented him also from abandoning a theory when it became increasingly questionable and even the novel methods predicted by the theory failed to work. Pauling would not disavow his claims of successful synthesis of specific artificial antibodies based on his 1940 theory when they were conclusively shown to be irreproducible by others.

121. Ute Deichmann, "Data, Theory, and Scientific Belief in Early Molecular Biology: Pauling's and Crick's Conflicting Notions about the Genetic Determination of Protein Synthesis and the Solution to the 'Secret of Life,'" *HYLE—International Journal for Philosophy of Chemistry* 27 (2021), 25–46.

It should be added that a few years later, alternative template theories, according to which macromolecules, such as proteins and nucleic acids, were synthesized from their building blocks with the help of enzymes, rose to the fore and have remained very important in molecular biology. But these template theories were fundamentally different from that of Pauling and other immunochemists in that with these, the complementary sequences of the building blocks (amino acids or nucleotides) were selected by the sequences of the template strands, and not "instructed" by their molecular shape.

The ideas of simplicity and parsimony led other chemists to search for—and allegedly find—regularity and periodicity in protein (primary) structure, i.e., their linear chain of amino acids. The resulting marginalization of the amino acid sequence for function could be reconciled with Pauling's assumption that the three-dimensional shape of proteins and their associated functions were independent of sequence. However, it contradicted the increasingly accepted evidence that sequence was important for function and that biological specificity and biological information were based on intrinsic "aperiodicity" (Schrödinger) and sequence diversity.

5.2. Irreproducibility in protein chemistry

The discovery of novel specific proteins and the experimental generation of such proteins, such as enzymes and antibodies, have been longstanding goals in the history of chemistry and biochemistry. Research to this end appears to have been especially prone to irreproducible experiments, as the case of Pauling and the vast amount of citations of his paper on antibody generation illustrates. A few prominent other cases are briefly mentioned here.

An early example is Emil Abderhalden's assertion, in 1910 and for forty years thereafter, of the existence of specific defense enzymes, i.e., protective enzymes in the blood of animals and humans that could be used as diagnostic tools in a wide variety of diseases and abnormalities. The existence of such enzymes could not be demonstrated in experiments that were repeatable by others. The case of Abderhalden exemplifies the transition from self-suggestion to the deception of others and finally to outright fraud.[122] The article in which Benno Müller-Hill and the present author exposed the problems of Abderhalden's research received many responses, mostly from biochemists who were

122. Ute Deichmann and Benno Müller-Hill, "The Fraud of Abderhalden's Enzymes," *Nature* 393 (1998): 109–11.

active during this period of time. Among them was biochemist Peter Karlson, who wrote: "It is commendable that you and B. Müller-Hill carried them [the defense enzymes] to their grave. I still experienced the end of their flourishing period, and the controversies about them. Many biochemists were of the opinion that there had to be 'something to it' and yet, everything was mass suggestion."[123]

The lack of noticeable specificity of the antibodies that purportedly had been produced *de novo* was the major reason that Pauling's experiments could not be reproduced by others. A similar problem has also arisen in the more recent research that allegedly succeeded in the synthesis of specific catalytic antibodies—a revolutionary claim, because antibodies usually bind ligands; they do not catalyze reactions. First published by the renowned and aforementioned (see Section 4.4) American chemist Peter Schultz,[124] the idea of catalytic antibodies for the generation of tailor-made biological catalysts with extraordinary specificities received wide attention and praise. However, later on, doubts began to be expressed with regard to the specificity of the catalysis, and in 1996 it was shown that normal blood proteins, i.e., serum albumins—that were called, "familiar 'off-the-shelf' proteins"—catalyze the same reaction at similar rates as the so-called catalytic antibodies.[125] Schultz some years later stopped working on catalytic antibodies.

In 1993, M. Zouhair Atassi and Taghi Manshouri claimed to have succeeded in synthesizing artificial enzymes with their artificial enzyme system, "pepzyme," in which they called into question received views on enzymes.[126] This was a revolutionary claim because despite an increasing understanding of the structure and function of enzymes, the synthesis of artificial enzymes with

123. Peter Karlson in letter to author, June 1998.

124. Peter G. Schultz, "Catalytic Antibodies," *Angewandte Chemie* International Edition 28, no. 10 (1989): 1283–95. See also Peter G Schultz and Richard A. Lerner, "From Molecular Diversity to Catalysis: Lessons from the Immune System," *Science* 269, no. 5232 (1995): 1835–42.

125. Florian Hollfelder, Anthony J. Kirby, and Dan S. Tawfik, "Off-the-Shelf Proteins that Rival Tailor-Made Antibodies as Catalysts," *Nature* 383 (1996): 60–63.

126. M. Zouhair Atassi and Taghi Manshouri, "Design of Peptide Enzymes (Pepzymes): Surface-simulation Synthetic Peptides that Mimic the Chymotrypsin and Trypsin Active Sites Exhibit the Activity and Specificity of the Respective Enzyme," *Proceedings of the National Academy of Sciences of the United States of America* 90 (1993): 8282–86; cited in Anthony J. Kirby, "Enzyme—Mechanismen, Modellreaktionen und Mimetica," *Angewandte Chemie* 108, no. 7 (1996): 770–90. The article was published without reviews because Atassi was a member of the American Academy of Sciences (Chaim Gilon, conversation with author, Jerusalem, 10 Apr 2019).

a catalytic capacity close to that of natural enzymes has not yet been achieved. Many researchers tried to reproduce the synthesis of these alleged artificial enzymes. But all attempts failed.[127]

The case of Pauling and some of the above-mentioned cases suggest that in addition to science-political factors, personal factors and subjective preferences of theories may have contributed to the frequent occurrence of questionable research and irreproducible experiments in the field of protein chemistry.[128] These cases have to be distinguished from those that are at the center of the discussion of irreproducibility in the medical sciences, social sciences, and in psychology today that is mainly related to problems in using statistics.[129] Statistical flaws, however, were and are not at the center of the irreproducibility of the work in experimental chemistry by Abderhalden, Pauling, Atassi, or Schultz.

In most of these cases, template models were proposed for creating specificity through protein-ligand interactions, a fact indicating that these models have remained attractive to researchers. The idea of specificity being transmitted directly by imprinting a three-dimensional shape on proteins was and is aesthetically pleasing and subjectively more appealing than the idea of a random genetic determination of amino acid sequences and subsequent selection of the proteins in question. This was pointed out by the immunochemists César Milstein (who shared the 1984 Nobel Prize in Physiology or Medicine with Niels Jerne and Georges Köhler) and Michael Neuberger, according to whom the "attraction of the learning process is clearly manifested in the early (instructive) theories of the origin of antibodies whereby the antigen was proposed to function as a template directing the final shape of the combining site of the specific antibody (e.g., Pauling, 1940). Perhaps that is why instructive theories remained popular for a long time, although they were based on misguided assumptions and inaccurate

127. Kirby, "Enzyme—Mechanismen" (ref. 126).

128. This is in addition to the widely discussed structural problems in science, such as the pressure to publish or the directing of funding toward potentially economically promising research. For an analysis of the social and political reasons for the spread of questionable research, irreproducible experiments, and fraud in protein research in Germany, 1920–1950, such as the priority funding of work that promised rapid practical application and economic success and the strengthening of scientific authorities during the Nazi era, see: Ute Deichmann, "Vertrauen, Betrug und Politik: Proteinforschung in Deutschland während der NS-Zeit," in *Expedition in die Wissenschaft* (Weinheim: Wiley-VCH, 2006), 21–37; Deichmann, "Proteinforschung" (ref. 101).

129. John P. A. Ioannidis, "Why Most Published Research Findings Are False," *PLOS Medicine* 2 (2005): 696–701.

predictions."[130] Similarly, in chemical technology, template-related molecular imprinting is cited, even today, with reference to Pauling. It seems that the concept of "mass suggestion" (see above) can also help understand this phenomenon.

It should be added that in all the cases presented in this article, the papers promised lucrative medical and industrial applications through revolutionary findings in protein chemistry. Specific proteins, such as enzymes, are crucial functional elements in organisms and their synthesis would tremendously impact applied biomedical research. Therefore, the specificity of the synthesized proteins—antibodies, enzymes—was a key for their successful application, and it was the lack of their specificity that characterized all the irreproducible papers discussed here.

The great prestige of many of the authors of the questionable papers certainly contributed to the long-standing popularity of their claims. Pauling, Schultz, and at the time in Germany, Abderhalden, were renowned researchers, a fact that contributed to critical questions being suppressed and refutations not published (though there were exceptions; see Section 2.3). Mention of a scientific authority in one's publication is as a rule beneficial, regardless of whether the cited work has been confirmed or not, especially when the authors did not admit their failure or retract their papers. Article retractions only began to become common in the 1980s.[131] But retracted papers, too, sometimes get cited in respectable scientific publications.[132]

An often overlooked consequence of not acknowledging the fact that papers turn out not to be reproducible is the harm inflicted on the practice of colleagues and students. For many years, aspiring young scientists who looked up to their mentors wasted their energies and material resources on research within the framework of what was believed to be a promising theory or successful new method, but which turned out to be questionable or completely

130. César Milstein and Michael S. Neuberger, "Maturation of the Immune Response," *Advances in Protein Chemistry* 49 (1996): 451–85.

131. Michael L. Grieneisen and Minghua Zhang,"A Comprehensive Survey of Retracted Articles from the Scholarly Literature," *PLOS ONE* 7, no. 10 (2012): e44118. https://doi.org/10.1371/journal.pone.0044118; "The Retraction Watch Database," Version: 1.0.6.0, http://retractiondatabase.org/RetractionSearch.aspx (accessed 14 Jun 2020).

132. Jaime A. Teixeira Da Silva and Judit Dobránszki, "Highly Cited Retracted Papers," *Scientometrics* 110, no. 3 (2017): 1653–61; R. Grant Steen, "Retractions in the Scientific Literature: Is the Incidence of Research Fraud Increasing?" *Journal of Medical Ethics* 37, no. 4 (2011): 249–53.

untenable.[133] Irreproducible scientific beliefs in fashionable fields and promoted by prominent scientists sometimes have a long life and seriously impact scientific practice.

The example of Pauling shows that the inclusion of subjective decisions and beliefs into scientific theories runs the risk of leading an unwary researcher astray. However, it should not be forgotten that subjectivity and commitment to scientific beliefs were also pre-requisites for the generation of most of the seminal theories and concepts in the history of modern biology, including the generation of Pauling's concepts of complementarity and molecular specificity.

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133. The consequences for the research community of scientific misconduct and irreproducibility have been highlighted in Ulrich Charpa and Ute Deichmann, "Vertrauensvorschuß und wissenschaftliches Fehlhandeln—Eine reliabilistische Modellierung der Fälle Abderhalden, Goldschmidt, Moewus und Waldschmidt-Leitz," *Berichte zur Wissenschaftsgeschichte* 27 (2004): 187–204.