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The 102 Most-Cited Life-Sciences Publications in the New 1945-1954 *Science Citation Index*. Part 2. Wonder Drugs, Cell Biochemistry, Separation Techniques Highlight Major Trends of Post-World War II Decade

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This essay concludes a two-part examination of 102 highly cited papers and books in the life sciences, 1945-1954. In this part, the five most-cited works are featured, along with some highlights and key developments of the decade—notably, work in endocrinology, protein structures, and separation techniques. The contributions of Nobel laureates from this era are also examined. As in several previous studies, this group of highly cited papers demonstrates the critical importance of methods and techniques in speeding scientific progress.

Wonder Drugs, Cell Biochemistry, Separation Techniques Highlight Major Trends of Post-World War II Decade

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In the first part of this essay, the list of 102 most-cited papers from the 1945-1954 *Science Citation Index®* cumulation was presented and discussed. In this second part Bernard Dixon discusses the major developments of the decade as revealed by citation analysis.

The Top Five

By a considerable margin, the most heavily cited life-sciences paper between 1945 and 1954 was one published in the *Journal of Biological Chemistry* by American biochemist Cyrus Hartwell Fiske with his colleague Y. SubbaRow as far back as 1925. Describing a colorimetric method for determining the amount of phosphorus in solutions, it was cited about 1,465 times (not to mention the countless occasions when it has appeared in doctoral theses and other documents). Fiske joined the faculty of Harvard Medical School in 1918 and served as professor of biochemistry there from 1935 to 1957, when he became professor emeritus. He discovered the cellular energy storage compound adenosine triphosphate, and pioneered the use of liver extracts to treat pernicious anaemia long before the introduction of vitamin B_{12} (whose isolation is also recorded in the Bibliography that appeared in Part 1[1]). But many biochemists and laboratory technicians today will recall his name largely as a result of the time-saving "Fiske and SubbaRow" assay.

Equally familiar to countless researchers and students will be G.W. Snedecor's *Statistical Methods Applied to Experiments in Agriculture and Biology*, the fourth edition of which was published in 1946 and was cited about 1,110 times between 1945 and 1954 (see W.G. Cochran's *Citation Classic*®[2] commentary on the fifth edition of 1956). It is followed by three further methods papers. These are: a description by R. Consden and colleagues (including Nobel laureate Archer J.P. Martin) of a paper-chromatographic technique for analysing proteins qualitatively (about 885 citations); L.J. Reed and H. Muench's paper explaining a simple method of estimating 50 percent endpoints (about 810 citations); and Norton Nelson's 1944 report on a modification of the Somogyi method for determining glucose (over 715 citations).

Nelson's technique is especially noteworthy because it originated in something of an emergency, when he and his brother Waldo, together with Arthur E. Mirsky and Samuel Rappaport, were working together in Cincinnati at the end of the 1930s. Their researches, on juvenile diabetes and other conditions, involved a considerable amount of glucose analysis. But, as Nelson later recalled in *Current Contents*®, "Somewhere along the line, perhaps about 1940, all refrigerators in all available institutes were jammed with specimens awaiting analysis, with which our staff, even with weekend work, were unable to keep abreast. Obviously something needed to be done and that was to improve the efficiency of glucose analysis, without losing the advantages of the Somogyi procedure."[3]

The answer proved to be a new arsenomolybdate reagent. This allowed M. Somogyi's existing copper reagents, then used for estimating glucose by a lengthy and tedious titration procedure, to be adapted and updated in a quick and reliable colorimetric method. "Within a few weeks our refrigerators, though not precisely empty, were clearly ready to receive more specimens," wrote Nelson, who later met Dr. Somogyi in St. Louis and was taken by his "predictable Hungarian charm."[3] But Somogyi, too, had devised a solution to the same problem, which was included with permission in the Nelson paper. Somogyi later wrote up his work in more detail in two papers published together. These papers, too, appear in the 1945-1954 listing (with about 235 and 410 citations, respectively).

Nobel Laureates

In addition to Philip S. Hench and Edward C. Kendall, discussed in Part 1,[1] there are 13 Nobel laureates in the list. One of them is the English biochemist Fred Sanger, who has won the chemistry prize on two occasions. In 1958, he was the sole recipient for his success in solving one of the greatest problems of twentieth-century biology—the structure of proteins, in particular, insulin. His 1945 paper on free amino-acid groups of insulin attracted over 360 citations. In 1980, nearly a quarter of a century after his first telegram arrived from Stockholm, Sanger shared the chemistry prize for work more properly described as molecular biology. He and Walter Gilbert were honoured for developing techniques which reduced drastically the amount of toil required to decode the sequences of nucleotide units along stretches of DNA. Together with what are now termed recombinant DNA procedures evolved by the third recipient, Paul Berg, this was the work that first made genetic engineering a practical possibility.

Sanger's research on insulin was greatly facilitated by a technique developed by the 1952 Nobel Prize winners in chemistry, the English chemist Martin and a fellow countryman, biochemist Richard L.M. Synge, while working together at the Wool Industries Research Association in Leeds in northern England. Their 1941 paper describing a new form of chromatography attracted over 235 citations. Partition chromatography appears to be disarmingly simple—as seen when a piece of filter paper is spotted with a solution containing several different substances, which are then separated as a liquid soaks through the paper and carries them different distances forward. Yet this technique transformed biomedical science, allowing new substances to be traced and isolated, new metabolic pathways to be charted, and fragments of large molecules such as proteins to be separated. Sanger's use of paper chromatography in 1955 to separate the amino acids from insulin—the first protein to be sequenced—was the first major demonstration of its immense power.

Next to penicillin, the most famed antimicrobial "magic bullet" must be streptomycin, whose inception virtually ended the terrors and miseries of tuberculosis. It was described in a 1944 paper co-authored by Albert Schatz, Elizabeth Bugie, and Selman A. Waksman, then working at Rutgers University, New Jersey. The paper was cited over 275 times in the period 1945-1954. The Russian-born Waksman, who became a naturalised American in 1916, had been studying antagonisms between different soil microorganisms for many years. Spurred by the early results with penicillin, he turned with his collaborators to a fungus, *Streptomyces griseus*, which he had isolated 28 years earlier and which proved to produce an extremely powerful antitubercular substance, which he named streptomycin.

In 1952, Waksman received the Nobel Prize in physiology or medicine. Then followed an unseemly dispute when Schatz filed a complaint demanding an order restraining Waksman from representing himself as the sole discoverer of streptomycin and demanding a share of royalties on the antibiotic. At this point, Waksman recalled a few years later (though without mentioning Schatz by name), "I felt a moral obligation to my associates, to my university and to all others who had supported my work throughout many years, who would have been involved in the drawn-out and unpleasant legal procedures. I reached the conclusion, not without great reluctance, that the situation made a speedy disposition of the lawsuit essential."[4] So Waksman agreed that Schatz should be legally recognized as co-inventor of streptomycin, and that he should receive $125,000 for the assignment of all foreign patent rights, and 3 percent of the royalties.

Another Nobelist in the list, the Swedish biochemist Arne Tiselius, received the 1948 chemistry prize "for his researches on electrophoresis and adsorption analysis, especially for his discoveries concerning the complex nature of the serum proteins." A pupil of The Svedberg, inventor of the ultracentrifuge, Tiselius brought electrophoresis to a considerable degree of perfection as a means of purifying colloids and high molecular weight substances. His primordial 1937 paper (which was cited about 235 times in 1945-1954) describes a new apparatus for achieving this.

Sharing the 1953 Nobel Prize in physiology or medicine were Hans Krebs and Fritz Lipmann—Krebs for discovering the citric acid cycle (which followed his work on the urea cycle mentioned in Part 1[1]) and German-born biochemist Lipmann for discovering coenzyme A and its importance in intermediary metabolism. The citric acid (or Krebs) cycle is a sequence of reactions through which energy is released from carbohydrates. It was disentangled by Krebs and his co-workers at the University of Sheffield, UK, principally in the years 1936-1937, Krebs having fled from Nazi Germany in 1933 when Hitler seized power. In June 1937, incidentally, *Nature* rejected Krebs's letter describing the citric acid cycle, and a full paper appeared less than two months later in *Enzymologia*.

Coenzyme A plays a key role in both the Krebs cycle and many other vital processes. Between 1927 and 1930 its discoverer, Lipmann, had worked in the same building as Krebs in Berlin at the Kaiser Wilhelm Institute for Biology—he under Otto Meyerhof and Krebs under Otto Warburg. Lipmann left Germany in 1931 for The Rockefeller Institute, New York, and later went to Copenhagen before emigrating permanently to the US in 1939. When the two met in Stockholm for the Nobel Prize ceremony in December 1953, Lipmann's wife Freda dubbed them "brothers-in-arms."[5]

A paper co-authored by two more Nobel laureates in the 1945-1954 listing again confirms the often under-estimated importance of experimental methods in science. Published by Stanford Moore and William H. Stein in 1948, it received about 400 citations over the 10-year period and described a photometric ninhydrin technique for use in chromatographing amino acids. Moore and Stein, who began working together at The Rockefeller Institute in the early 1940s, shared the 1972 Nobel Prize in chemistry with Christian B. Anfinsen of the National Institutes of Health, Bethesda, Maryland, for research which opened a new era in our understanding of the structure and function of enzymes.

Taking a different approach from that of Sanger, Moore and Stein decided to produce columns with which to separate the amino acids released when proteins are hydrolysed. By 1948, they had introduced a column, packed with starch granules, that was capable of resolving the amino acids from as little as one milligramme of hydrolysed protein. By modifying the existing ninhydrin reagent, which reacts with amino acids to give a purple color, they had also evolved a method of measuring the quantity of individual acids as they came off the column. Within 10 years, starch had been supplanted by ion-exchange resins, and the technique had been automated to produce the earliest of the modern generation of amino-acid autoanalysers.

These instruments reduced dramatically the amount of work involved in studying the primary structures of proteins, which in the case of some large molecules was so laborious as to be virtually impossible. Five years after Sanger's 1955 work on insulin, Moore and Stein used their new technique to determine the primary structure (the amino-acid sequence) of ribonuclease, an enzyme that splits ribonucleic acid (RNA) into its subunits. Ribonuclease was the first enzyme to be sequenced, and Anfinsen later determined how the amino-acid chain of the enzyme folds into its characteristic three-dimensional structure.

Another scientist who made a distinctive series of contributions to the emergence of a coherent picture of vital processes at the molecular level was the Rumanian-born cytologist George E. Palade. Honoured with the 1974 Nobel Prize in physiology or medicine alongside the Belgian-born Albert Claude and the Belgian (though English-born) Christian de Duve, Palade was the discoverer of the ribosome, the nucleoprotein particle on which proteins are assembled to match sequencing instructions encoded on messenger RNA. Co-authored with G.H. Hogeboom and Walter C. Schneider, Palade's 1948 description of another cell organelle, the mitochondrion, was cited on over 325 occasions over the 1945-1954 decade. In this paper, as in all of his work, Palade's signal contribution was to correlate the structure of subcellular particles with their functions. Palade settled in the US in 1946 and became a naturalised US citizen six years later. He worked in various capacities at The Rockefeller Institute until taking over the Department of Cell Biology at Yale University School of Medicine, New Haven, Connecticut, shortly before winning the Nobel Prize.

The final Nobel laureate in the 1945-1954 listing is renowned more for his clinical achievement than for a contribution to science *per se*. He is André Cournand, a French physiologist who went to live in the US in 1930 and became naturalised in 1941. Working with Dickinson Woodruff Richards at the Columbia University Division of Bellevue Hospital, New York, it was Cournand who was largely responsible for establishing cardiac catheterisation as a safe, routine technique.

In 1929, a young intern at a small provincial hospital near Berlin, Werner Forssmann, inserted a catheter into a vein in his left arm and threaded it into his heart.[6] But such was the danger in this heroic procedure that Forssmann was thrown out of his hospital by the distinguished surgeon Ernst Ferdinand Sauerbruch, and the technique did not gain wide acceptance. Twelve years later, however, Cournand and his colleague

H.A. Ranges published a paper in which they described modifications making the procedure safer and demonstrated that the pulmonary artery could be catheterised too. It was cited over 220 times between 1945 and 1954. Cournand developed an improved method of passing the tube into the vein, and showed that a tube made of woven material strengthened with plastic was sufficiently stiff to transmit pressures accurately but not so stiff that its introduction into the heart would be dangerous. In 1956, Cournand, Forssmann, and Richards shared the Nobel Prize in physiology or medicine, and the technique has now become a standard procedure for investigating heart malfunctions, as well as being used in research.

Other Highlights of the Decade

A "Nobel Prize that never was" is symbolised by the appearance in the 1945-1954 listing of the paper entitled "Studies on the chemical nature of the substance inducing transformation of pneumococcal types," published by Oswald T. Avery, Colin M. MacLeod, and Maclyn McCarty in 1944. A core paper to one of ISI®'s research fronts for 1987, as mentioned in Part 1,[1] this was a key contribution which paved the way for the discovery of the DNA double helix and thus for the development of modern molecular biology. Cited over 235 times over the decade, it showed for the first time that a particular chemical substance, DNA, plays a role in altering the hereditary make-up of an organism and that it is capable of reproducing itself precisely "in amounts far in excess of that originally added."[7] The paper was doubly impressive for the variety of experimental techniques which Avery and his Rockefeller Institute colleagues brought to bear on the problem.

Yet this primordial work was not recognized by the Nobel accolade. In his book *The Transforming Principle: Discovering That Genes Are Made of DNA*, McCarty suggests that strongly and publicly expressed scepticism from former collaborator Alfred E. Mirsky may have influenced the Nobel Prize committee.[8] At a Cold Spring Harbor Symposium in 1947 Mirsky argued vociferously against the proposition that DNA had been identified as the specific carrier of hereditary information. (Ironically, perhaps, a 1947 paper on nucleoprotein co-authored by Mirsky and A.W. Pollister received about 235 citations in 1945-1954, just short of the Avery paper.)

The official Nobel book *Nobel: The Man and His Prizes* states that Avery accomplished "one of the most important discoveries of modern biology" and that "the discovery, because of its far-reaching implications, aroused much interest." The text continues: "Avery was proposed for a Nobel prize. But doubts were also expressed, and the Nobel Committee found it desirable to postpone an award. Actually, Avery's finding was not accepted in all quarters until A.D. Hershey and M. Chase, in 1952, demonstrated that bacteriophage-DNA carries the viral genetic information from parent to progeny."[9] McCarty adds a significant rider: "They do not comment on the fact that Avery lived for three years after 1952."[8]

With over 280 citations over the decade, the paper in which D.D. Woods revealed the mode of action of the "sulpha drugs" might also have led to a Nobel Prize had it initiated an era in which improved antimicrobial agents could be designed for their specific actions against bacteria and other microorganisms. The sulphonamides, introduced following work by German bacteriologist Gerhard Domagk in the mid-1930s, were the first worthwhile antimicrobial drugs, and it was Woods, together with Sir Paul Fildes, who found out how they work. Para-aminobenzoic acid (PABA) is an essential nutrient for many bacteria, and the sulphonamides, whose structures closely resemble that of PABA, compete with it for a site on the enzyme that normally handles the nutrient.

There were high hopes that pharmacologists could exploit this "competitive inhibition" to block the uptake of other vital nutrients by disease-causing microbes, and to interfere with other aspects of microbial metabolism. But such hopes were largely disappointed. In practice, things have worked the other way around. From penicillin onwards, most antibiotics have been discovered more pragmatically, by screening likely producers. Only *afterwards* have they been used to reveal those metabolic processes that might have provided targets for rational attack.

Aside from books and papers devoted to methods, the remaining highly cited books and papers of the decade range over the entire landscape of biomedical science. The achievements recorded here include Leonor Michaelis and M.M.L. Menten's description of the equation which explains the rate of variation of enzyme-catalysed reactions in relation to the concentrations of reacting substances (over 235 citations); and the isolation of vitamin B_{12}, used to treat pernicious anaemia, by E.L. Rickes, Karl Folkers, and co-workers (about 225 citations). Two papers under the name of Edwin Bennett Astwood (about 235 and 225 citations) describe work which led to the use of thiouracil to treat exophthalmic goiter. Fuller Albright's *Harvey Lectures* on Cushing's syndrome attracted about 220 citations, as did his review with E.C. Reifenstein of the parathyroid glands and metabolic bone disease.

The Importance of Techniques

In addition to those already mentioned, 44 other papers are devoted solely or principally to methods. American enzymologist Van Rensselaer Potter of the University of Wisconsin, Madison, received about 515 citations for his paper with C.A. Elvehjem describing a new approach to tissue oxidations, and over 250 citations for a paper on the assay of respiratory enzymes, of which Schneider was principal author. A paper by Schneider on the estimation of nucleic acids (based on his doctoral dissertation under Potter as his supervisor) also received about 540 citations. Recalling this work in a *Citation Classic* essay 22 years later, Schneider confessed that his discovery rested in part on his misreading of one of Zacharias Dische's papers in the *Biochemisches Zeitschrift*.[10]

Schneider's paper appeared in the very same issue of the *Journal of Biological Chemistry* as one on nucleic acid determination by Gerhard Schmidt and S.J. Thannhauser—which also occurs in the 1945-1954 listing, with about 415 citations. Schneider spotted the strengths and weaknesses of both techniques, combined the best features of each, and published a further paper the following year.

Three other research workers widely known for their scientific achievements but emerging here as technical innovators are the US poliomyelitis vaccine pioneer Jonas E. Salk, French-born microbiologist René J. Dubos, and the Canadian biochemist and physician J.B. Collip. Salk's report on a method of titrating the hemagglutinating capacity of influenza virus was cited over 240 times. Dubos's work with B.D. Davis on the culture of tubercle bacilli was cited on about 240 occasions.

Collip is famed largely for his contributions to the discovery of ACTH and for isolating parathormone from the parathyroid gland and introducing it in the treatment of tetany. He also collaborated with Charles Best in purifying insulin. When the 1923 Nobel Prize in physiology or medicine went solely to their colleagues Frederick G. Banting and John J.R. MacLeod (who had played no active part in the work), Banting expressed his dissatisfaction by sharing his half of the money with Best, whereupon MacLeod gave a corresponding part to Collip. In the 1945-1954 listing, Collip received about 245 citations for a paper (with E.P. Clark as principal author) on the measurement of serum calcium.

In addition to Consden, A.H. Gordon, and Martin, whose work was discussed earlier and who were the joint authors of two papers (about 240 and 885 citations), the names of three other innovators in laboratory methods appear twice in the listing. Particularly familiar to biochemists is that of the Swedish chemist Otto Folin. In 1912 he reported new micromethods of determining urea, total nitrogen, and ammoniacal nitrogen, but appears here for his work on amino acids and blood analysis. Although Folin died in 1934, his two papers (the first co-authored with V. Ciocalteu and the second with H. Wu) received about 290 and 260 citations, respectively, in 1945-1954. G. Gomori registered over 260 and 330 citations for papers on the determination and distribution of phosphatase, while S.M. Partridge received about 225 and 550 citations for innovations in partition chromatography. Another notable name is that of the English geneticist and pioneer statistician Sir Ronald A. Fisher, who rated over 230 citations for a contribution on statistical methods for research workers.

Books

As well as those discussed previously, 12 books appear in the list. Especially significant is Hans Selye's *Stress: The Physiology and Pathology of Exposure to Stress*, which sets out his pioneering work on the "general adaptation syndrome." Selye unified our understanding of this condition, which is mediated by the hormones of the adrenal cortex, orchestrated by the pituitary gland, and includes energy mobilisation, shrinkage of the lymphatic organs, and inhibition of allergies. Although published only in 1950, the book had registered over 250 citations by the end of the decade up to 1954. Selye was born in Vienna and educated in Hungary, Czechoslovakia, France, and Italy before settling at McGill University, Montreal (where he was for a while a research student under Collip). He also received about 665 citations for a paper on adaptation published in 1946, which became the first paper ever subjected to citation analysis.[11]

Of the remaining books, the largest number of citations (over 480) was to C.L. Hull's *Principles of Behavior: An Introduction to Behavior Theory*, while Joseph Needham's classic *Biochemistry and Morphogenesis* was cited about 260 times. *Neoplastic Diseases: A Treatise on Tumors* by the American pathologist James Ewing, who pioneered radium treatment and described a sarcoma usually affecting the shafts of long bones, received over 290 citations. One of the books, by D.H. Bergey and R.S. Breed, *Manual of Determinative Bacteriology*, is the bacteriologist's "bible," successive editions of which present the currently accepted classification of bacteria into orders, families, genera, and species. The sixth edition, published in 1948, rated about 255 citations. Only one of the listed books is in a language other than English—L. Lison's *Histochimie Animale*, which was published in Paris in 1936 and received about 220 citations.

Conclusion

In terms of overall trends, ISI's scrutiny of citations in the life-sciences literature of 1945-1954 has thrown up some unexpected findings and underlined a crucial but often underestimated aspect of research. One surprise is the fact that of the 102 items in the list, cancer is the subject of only 2—the books by Ewing and R.A. Willis. This is a reminder, perhaps, of how little was known about cancer and carcinogenesis in the first half of this century. Real scientific understanding of malignant disease (whether through molecular biological research into oncogenes or epidemiological studies such as those linking smoking with carcinoma of the lung) has developed much more recently. Somewhat surprising too is the small number of papers—10 at most—which are concerned with communicable diseases. Yet the listing does include key papers on three of the major highlights (arguably *the* major highlights) of antimicrobial warfare—the successive introduction of sulphonamides, penicillin, and streptomycin.

The major themes emerging from the study are the maturing of endocrinology as a scientific discipline and the laying of the foundations of molecular biology. No less than 21 of the highly cited items, including those by Selye and by Hench and his co-workers, concern various aspects of hormones, their natural functions, and their therapeutic potential. It was in 1936 that the American biochemist Edward Doisy proposed the criteria by which endocrinology could become an established area for scientific investigation. The items in the 1945-1954 citation study clearly reflect that process of authentication and maturation.

The second largest group of items (13 in all) describe various aspects of investigations into proteins and nucleic acids—the macromolecules upon which life depends and whose study is the centrepiece of molecular biology. Particularly noteworthy are the high scores recorded by Moore and Stein and by Martin and Synge (and Consden, Gordon, and Martin) for their innovations in chromatography. As confirmed by this citation study, the work of the 1952 Nobel laureates Martin and Synge and the 1972 laureates Moore and Stein was enormously influential. It was crucial to the later achievements of Sanger and others in determining the structures of key enzymes and other macromolecules. All the more strange, then, that Horace Freeland Judson's monumental and otherwise highly impressive history of molecular biology, published in 1979, fails to mention any one of those four innovators in technique.[12] As vividly demonstrated by those individuals, and by the emergence of methods as the basis of about half of the most heavily cited papers in 1945-1954, science is by no means all about theories, speculation, and purely intellectual revolution.


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