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A Retrospective Look at the Discovery of the Genetic Role of DNA

To Josh
with warm regards
Mac

By Maclyn McCarty

Erwin Chargaff's base-pairing equivalences (A=T and G=C) were of central importance for evaluating the merits of competing models of DNA structure. The so-called 'Chargaff Rules' were decisive in rejecting most models, and were ultimately definitive for the editing process by which Watson and Crick deduced their double helical model of DNA structure. In his penetrating autobiographical memoir, *Heraclitean Fire,* Chargaff used the following words to describe the profound personal impact that this month's 50 Years Ago report had for work in his laboratory: *Early in 1944 somebody told me about a paper he had seen in the* Journal of Experimental Medicine. *This was the celebrated paper by Oswald T. Avery, Colin MacLeod, and Maclyn McCarty . . . and these are the words with which they concluded their paper: "The evidence presented supports the belief that a nucleic acid of the deoxyribose type is the fundamental unit of the transforming principle of Pneumococcus Type III." It is difficult for me to describe the effect that this sentence, and the beautiful experimentation that had given rise to it, had on me . . . . Seldom has more been said in so few words . . . . I decided to relinquish all that we had been working on . . . .To the scientist nature is as a mirror that breaks every 30 years; and who care about that broken glass of past times? . . .The new finding made it, therefore, extremely probable that the genes contained, or consisted of, DNA. I believe that few people now would deny that this is one of the most important discoveries in biology.*

. . . At the time the publication appeared, however, most people—including the Nobel Prize Committee, as it was then constituted—did not pay the slightest attention to it. Those who should have known were all too busy spinning their own tops through the corridors of power.

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The paper that appeared 50 years ago in the February 1944 issue of *The Journal of Experimental Medicine*, reporting that the substance inducing the transformation of pneumococcal types is deoxyribonucleic acid (1), was most unusual by today's standards in that it covered work that had been carried out during the previous 10 years. This is certainly not likely to happen in the current era of grant-supported research. The most recent paper on the subject of transformation from the Avery laboratory had been published in 1933 by J. Lionel Alloway (2). This was his second paper describing transformation using cell-free extracts of encapsulated pneumococci as the transforming agent. Thus, by 1933 the information was available that extracts containing the soluble constituents of encapsulated pneumococci could cause rough, unencapsulated pneumococci derived from a different type to produce a capsule composed of the specific polysaccharide characteristic of the organism from which the extract was prepared. It is remarkable that no investigators outside the Avery laboratory were motivated by these findings to look for the identity of the active substance in the extracts, but I know of no evidence that any other efforts were made.

The work reported in the 1944 paper included the studies begun by Colin M. MacLeod when he arrived at the Rockefeller Institute for Medical Research in the summer of 1934. Research on transformation was his major laboratory activity for the next 3 years, and it was directed at several different aspects of the problem. He derived the rough strain, R36A, from an encapsulated type II pneumococcus during that first summer. Because of its improved susceptibility to transformation and its stability as an R form, it was used throughout the subsequent years. His other work improved the reliability of the rather uncertain test system for transforming activity and initiated approaches to fractionating the extracts. However, by 1937 he was too far from his goal of identifying the active component, and the work was almost completely suspended in order to pursue more productive activity.

This was planned as only a temporary hiatus, and in the fall of 1940 he and Avery resumed the search. Avery has been involved as an interested observer and advisor during MacLeod's early work but he had not participated actively in the research because of ill health. However, he had continued to be obsessed with the importance of discovering the identity of the transforming principle, and after his recovery from Graves' disease he was eager to get back to the bench to do active research on the problem.

At the outset of this renewed effort, some of the old problems continued to retard progress: variability in the activity of the extracts prepared by the Alloway method of lysing the pneumococcal cells with bile salt, and unreliability of the test system. New information began to emerge, however. In January 1941 it was first discovered that DNA was a component of the extracts, although the presence of large amounts of RNA had been known for some time. In March, the extraction procedure had been changed by heat-killing the cells at 65°C immediately after harvesting to inactivate the enzyme of the pneumococcus that destroyed transforming activity. By extracting the heated cells with a higher concentration of bile salt as a detergent, material could be obtained consistently that was more active than that obtained by lysis of living cells.

A series of experiments designed to fractionate extracts prepared by this new method were carried out in the spring of 1941, but on July 1, MacLeod departed to become chairman of the department of microbiology at the New York University School of Medicine. By pure chance, I moved in the opposite direction from N.Y.U. to the Avery laboratory at Rockefeller in September, and by the end of the month found myself working on transformation at the bench at Avery's side.

It was an exhiliarating experience, even with the ups and downs that were inherent in work on transformation. One didn't have to work long with the phenomenon and think about what was going on in the process of transformation before becoming convinced that it was of primary importance to get to the heart of the problem and identify the active substance. Clearly something akin to the transfer of genetic information was taking place. The likelihood that we were dealing with DNA as the active transforming agent came out of a series of observations during the ensuing months, and ultimately led to the development of procedures for the purification of DNA, involving the removal from the extracts of protein, RNA, type III polysaccharide, and other components. The final products had higher activity than any material obtained previously and their purity was confirmed by a variety of procedures. After several

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repetitions of this purification process, we were ready in the summer of 1943 to begin writing up the work, and we completed the process and submitted the manuscript for publication on November 1.

The paper, with the full title: "Studies on the Chemical Nature of the Substance Inducing Transformation of Pneumococcal Types. Induction of Transformation by a Desoxyribonucleic Acid Fraction Isolated from Pneumococcus Type III" appeared on February 1, 1944 (1). That it was something less than an immediate sensation is in no small part due to its appearance at a time in the course of World War II, some 4 months before the onset of the Normandy invasion, when most potential readers of the paper were otherwise engaged. There were other factors involved, however, such as the readership of the journal, which did not include geneticists and other biologists outside the field of experimental medicine. But even those who knew about the paper had reasons for skepticism about the findings. For biochemists there was the prevailing view that nucleic acids were too limited in diversity to possess biological specificity; and geneticists did not consider bacteria, with their apparent lack of sex, nuclei, and mitosis, as belonging in the mainstream of biology. There were, of course, many who accepted the validity of the evidence presented and interpreted the paper as a revolutionary advance, among them those who based their own work on the premise that we were correct, as noted below.

The view most widely expressed by the skeptics was that it could not be DNA because nucleic acids are all alike. They proposed that a small amount of highly active protein contaminating our DNA preparations was responsible for their biological activity. This was a difficult criticism to answer. We had progressively eliminated protein to below the limit of our methods of detection, without any loss of transforming activity, but the possibility of residual protein in amounts that could be significant in view of Avogadro's number remained. We had shown that crude enzyme preparations containing DNase would rapidly destroy transforming activity, whereas proteolytic enzymes were without effect. However, we did not have a purified DNase to test at the time, and this became one of my principal research projects after publication of the initial paper. In due course I prepared a purified pancreatic DNase (3) that in nanogram amounts would rapidly inactivate the transforming substance (4), showing at the very least that if a protein were involved, its function depended on the presence of intact DNA. The work of Hotchkiss (reviewed in ref 5), who followed me in the Avery laboratory when I departed in 1946, added to the evidence by further purification of transforming DNA and, more important, showed that the amino acids found in hydrolysates of his purified DNA were limited almost entirely to a single example, glycine, derived from the degradation of adenine.

Thus, the ghost of a protein contaminant as the transforming agent was pretty well laid to rest in the latter half of the 1940s. In work that broadened the implications of the phenomenon, Hotchkiss established that characters other than capsule synthesis could be transferred by pneumococcal DNA, including antibiotic resistance and synthesis of an enzyme (5, 6). Other workers described additional transferable characters, thus adding to the evidence for broader genetic significance. In addition, DNA transformation was shown to occur in bacterial species other than pneumococci.

A notable advance in another area came from the work of Chargaff (7), who had been stimulated to change the focus of his research to nucleic acid chemistry after reading the 1944 paper. He showed that DNAs from different sources were far from all alike in composition, as had been assumed. Furthermore, he established the importance of base-pairing (A-T and G-C), which provided a seminal clue in the solution of the structure of DNA (7).

In effect, within a relatively few years after the publication of the 1944 paper, the basis for the doubts and skepticism about the identification of the transforming substance as DNA had been proved to be untenable. This had occurred before the report of the Hershey-Chase experiment in 1952 (8), which could have been challenged by the same objections that were raised originally to the identification of the transforming principle as DNA. The great impact of this experiment derived from its description of another experimental model, completely different from bacterial transformation, in which information was carried by DNA.

It it also evident that the selection of DNA for their structural studies by Crick and Watson (9, 10) was dependent on the evidence for its genetic role that came from the pneumococcal transformation work. Thus, it is difficult to agree with those who argue that the paper had little effect. A great deal of progress had been made in the decade after the first report that not only served to verify the genetic role of DNA but also set in motion the steps required for the integration of this information into all of biological research.

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