TRANSFORMING BIOLOGY

Maclyn McCarty, M.D.
The Rockefeller University, New York, New York, U.S.A.

In view of the recent upsurge in the successful application of molecular approaches to a wide range of medical problems, it is sobering to recall that the chemical nature of the gene was unknown at the time that the United States became involved in World War II. The flowering of molecular medicine is a phenomenon of the late 20th century.

One of the notable aspects of the experimental work that led to the discovery of the genetic role of DNA is that it was directed toward a medical problem, bacterial pneumonia, and was carried out from beginning to end by medical microbiologists. It is gratifying to me, as a survivor among those engaged in these experiments, that the results are now paying off so handsomely in research on the major medical problems of the age.

The story began in England in the 1920s with the work of Fred Griffith, who had been impressed by the fact that sputum samples from pneumonia patients, submitted to his laboratory for diagnosis, often yielded as many as four or five different specific capsular types of pneumococci. He considered that interchangeability of type was as likely as the separate acquisition of so many different organisms and carried out experiments to test his idea. Out of this came the phenomenon of transformation of pneumococcal types. The experiments were carried out in the highly susceptible mouse and involved the injection of a bolus of heat-killed pneumococci of one capsular type together with a small inoculum of living unencapsulated organisms derived from a different capsular type. In many instances, the mice died of infection with organisms bearing capsules of the type of the heat-killed cells. This was repeated with a variety of pairs of strains to establish the generality of the phenomenon, with extensive controls to assure that the heat-killed cells were unquestionably dead.

Griffith published his surprising results in 1928. They were fully confirmed by Fred Neufeld at the Koch Institute in Berlin later in the same year and by Martin Dawson in the Avery laboratory at The Rockefeller Institute in New York by 1930. It is interesting that nearly all of the subsequent work on the problem emerged from the Avery laboratory, while other groups were not motivated to explore the nature of pneumococcal transformation. Dawson, in experiments continued after he moved to Columbia University, succeeded in eliminating the mouse by carrying out the transformation reaction in the test tube. The next important step was taken by Lionel Alloway in the Avery lab when he prepared cell-free extracts of pneumococci to replace heat-killed cells in the transforming system.

Thus, by 1934 a potential system for a better analysis of the nature of transformation was at hand. It was, however, a maddeningly unreliable and frustrating system for carrying out quantitative assays. Colin MacLeod devoted much of his efforts upon joining the Avery lab in 1934 to improving the system and made a series of changes that enhanced its reliability to some degree. He also applied a number of enzymatic and biochemical procedures to the cell-free extracts, showing, for example, that removal of protein by the chloroform process had no effect on transforming activity. Progress toward the goal of identifying the active component of the extracts was slow, however, and in 1937 the project was temporarily put aside to make way for more productive research.

Throughout these early years, research on transformation continued to be motivated by its possible bearing on the problem of pneumococcal pneumonia. In their annual report in the spring of 1936, Avery and MacLeod discussed the work from this point of view without mentioning the possibility of broader implications. Nevertheless, I believe that by this time the possible genetic implications of transformation were in their minds. One could hardly work with and

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think about the process without noting that it resembled the transfer of genetic information.

When Avery and MacLeod resumed work on the problem in October 1940, they continued to be faced by difficulties, most notably the unpredictable variation in the potency of extracts prepared by the Alloway method. They pushed ahead with the work, however, and in January 1941 discovered that their active extracts contained DNA. The limited knowledge of bacterial biochemistry had not yet made it obvious that it was certain to be present. In March, chiefly at the instigation of Avery, they discarded the Alloway method of preparing extracts and consistently obtained much more active material by extracting pneumococcal cells that were first heat-killed, thus avoiding exposure to autolytic enzymes. With this improved process, they were accumulating active extracts and testing fractionation procedures until July, when MacLeod departed to assume the chairmanship of the Department of Microbiology at New York University.

My arrival in the Avery lab as a postdoctoral fellow 2 months later was purely a matter of chance, but, by the end of September, I had joined Avery at the bench working on transformation. My first project came from a finding of the previous spring suggesting that the capsular polysaccharide in the extracts, which separated out in fibrous strands on alcohol precipitation, was difficult to separate from the transforming substance and thus might be essential as a template in the synthesis of new polysaccharide by the transformed cells. My task was to determine whether complete hydrolysis of the polysaccharide with the Dubos SIII enzyme caused a loss in transforming activity. It did not, and this led to preparing extracts that lacked detectable capsular polysaccharide and showing that they still contained material that yielded fibrous alcohol precipitates. This fibrous material was identified as DNA and the direction of the search was set.

A variety of procedures, including further fractionation, ultracentrifugal analysis, and enzymatic tests, were applied to establish that transforming activity was associated with DNA. Purified type III pneumococcal DNA preparations was made from several large batches of organisms and their purity confirmed on detailed analysis. Each of these preparations induced transformation in nanogram quantities.

By the summer of 1943, the accumulated information from years of research had finally made it clear that the transforming principle was DNA, and the time had come to write up the results. The manuscript was submitted on November 1 and appeared in the *Journal of Experimental Medicine* February 1, 1944.