

= Avery ? MacLeod ? McCarty experiment =

The Avery ? MacLeod ? McCarty experiment was an experimental demonstration , reported in 1944 by Oswald Avery , Colin MacLeod , and Maclyn McCarty , that DNA is the substance that causes bacterial transformation , in an era when it had been widely believed that it was proteins that served the function of carrying genetic information (with the very word protein itself coined to indicate a belief that its function was primary) . It was the culmination of research in the 1930s and early 1940s at the Rockefeller Institute for Medical Research to purify and characterize the " transforming principle " responsible for the transformation phenomenon first described in Griffith 's experiment of 1928 : killed *Streptococcus pneumoniae* of the virulent strain type III @-@ S , when injected along with living but non @-@ virulent type II @-@ R pneumococci , resulted in a deadly infection of type III @-@ S pneumococci . In their paper " 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 " , published in the February 1944 issue of the *Journal of Experimental Medicine* , Avery and his colleagues suggest that DNA , rather than protein as widely believed at the time , may be the hereditary material of bacteria , and could be analogous to genes and / or viruses in higher organisms .

= = Background = =

With the development of serological typing , medical researchers were able to sort bacteria into different strains , or types . When a person or test animal (e.g. , a mouse) is inoculated with a particular type , an immune response ensues , generating antibodies that react specifically with antigens on the bacteria . Blood serum containing the antibodies can then be extracted and applied to cultured bacteria . The antibodies will react with other bacteria of the same type as the original inoculation . Fred Neufeld , a German bacteriologist , had discovered the pneumococcal types and serological typing ; until Frederick Griffith 's studies bacteriologists believed that the types were fixed and unchangeable from one generation to the next .

Griffith 's experiment , reported in 1928 , identified that some " transforming principle " in pneumococcal bacteria could transform them from one type to another . Griffith , a British medical officer , had spent years applying serological typing to cases of pneumonia , a frequently fatal disease in the early 20th century . He found that multiple types ? some virulent and some non @-@ virulent ? were often present over the course of a clinical case of pneumonia , and thought that one type might change into another (rather than simply multiple types being present all along) . In testing that possibility , he found that transformation could occur when dead bacteria of a virulent type and live bacteria of a non @-@ virulent type were both injected in mice : the mice would develop a fatal infection (normally only caused by live bacteria of the virulent type) and die , and virulent bacteria could be isolated from such infected mice .

The findings of Griffith 's experiment were soon confirmed , first by Fred Neufeld at the Koch Institute and by Martin Henry Dawson at the Rockefeller Institute . A series of Rockefeller Institute researchers continued to study transformation in the years that followed . With Richard H.P. Sia , Dawson developed a method of transforming bacteria in vitro (rather than in vivo as Griffith had done) . After Dawson 's departure in 1930 , James Alloway took up the attempt to extend Griffith 's findings , resulting in the extraction of aqueous solutions of the transforming principle by 1933 . Colin MacLeod worked to purify such solutions from 1934 to 1937 , and the work was continued in 1940 and completed by Maclyn McCarty .

= = Experimental work = =

Pneumococcus is characterized by smooth colonies and has a polysaccharide capsule that induces antibody formation ; the different types are classified according to their immunological specificity .

The purification procedure Avery undertook consisted of first killing the bacteria with heat and extracting the saline @-@ soluble components . Next , the protein was precipitated out using

chloroform and the polysaccharide capsules were hydrolyzed with an enzyme . An immunological precipitation caused by type @-@ specific antibodies was used to verify the complete destruction of the capsules . Then , the active portion was precipitated out by alcohol fractionation , resulting in fibrous strands that could be removed with a stirring rod .

Chemical analysis showed that the proportions of carbon , hydrogen , nitrogen , and phosphorus in this active portion were consistent with the chemical composition of DNA . To show that it was DNA rather than some small amount of RNA , protein , or some other cell component that was responsible for transformation , Avery and his colleagues used a number of biochemical tests . They found that trypsin , chymotrypsin and ribonuclease (enzymes that break apart proteins or RNA) did not affect it , but an enzyme preparation of " deoxyribonucleodepolymerase " (a crude preparation , obtainable from a number of animal sources , that could break down DNA) destroyed the extract 's transforming power .

Follow @-@ up work in response to criticism and challenges included the purification and crystallization , by Moses Kunitz in 1948 , of a DNA depolymerase (deoxyribonuclease I) , and precise work by Rollin Hotchkiss showing that virtually all the detected nitrogen in the purified DNA came from glycine , a breakdown product of the nucleotide base adenine , and that undetected protein contamination was at most 0 @. @ 02 % by Hotchkiss 's estimation .

= = Reception and legacy = =

The experimental findings of the Avery ? MacLeod ? McCarty experiment were quickly confirmed , and extended to other hereditary characteristics besides polysaccharide capsules . However , there was considerable reluctance to accept the conclusion that DNA was the genetic material . According to Phoebus Levene 's influential " tetranucleotide hypothesis " , DNA consisted of repeating units of the four nucleotide bases and had little biological specificity . DNA was therefore thought to be the structural component of chromosomes , whereas the genes were thought likely to be made of the protein component of chromosomes . This line of thinking was reinforced by the 1935 crystallization of tobacco mosaic virus by Wendell Stanley , and the parallels among viruses , genes , and enzymes ; many biologists thought genes might be a sort of " super @-@ enzyme " , and viruses were shown according to Stanley to be proteins and to share the property of autocatalysis with many enzymes . Furthermore , few biologists thought that genetics could be applied to bacteria , since they lacked chromosomes and sexual reproduction . In particular , many of the geneticists known informally as the phage group , which would become influential in the new discipline of molecular biology in the 1950s , were dismissive of DNA as the genetic material (and were inclined to avoid the " messy " biochemical approaches of Avery and his colleagues) . Some biologists , including fellow Rockefeller Institute Fellow Alfred Mirsky , challenged Avery 's finding that the transforming principle was pure DNA , suggesting that protein contaminants were instead responsible . Although transformation occurred in some kinds of bacteria , it could not be replicated in other bacteria (nor in any higher organisms) , and its significance seemed limited primarily to medicine .

Scientists looking back on the Avery ? MacLeod ? McCarty experiment have disagreed about just how influential it was in the 1940s and early 1950s . Gunther Stent suggested that it was largely ignored , and only celebrated afterwards ? similarly to Gregor Mendel 's work decades before the rise of genetics . Others , such as Joshua Lederberg and Leslie C. Dunn , attest to its early significance and cite the experiment as the beginning of molecular genetics .

A few microbiologists and geneticists had taken an interest in the physical and chemical nature of genes before 1944 , but the Avery ? MacLeod ? McCarty experiment brought renewed and wider interest in the subject . While the original publication did not mention genetics specifically , Avery as well as many of the geneticists who read the paper were aware of the genetic implications ? that Avery may have isolated the gene itself as pure DNA . Biochemist Erwin Chargaff , geneticist H. J. Muller and others praised the result as establishing the biological specificity of DNA and as having important implications for genetics if DNA played a similar role in higher organisms . In 1945 , the Royal Society awarded Avery the Copley Medal , in part for his work on bacterial transformation .

Between 1944 and 1954 , the paper was cited at least 239 times (with citations spread evenly through those years) , mostly in papers on microbiology , immunochemistry , and biochemistry . In addition to the follow @-@ up work by McCarty and others at the Rockefeller Institute in response to Mirsky 's criticisms , the experiment spurred considerable work in microbiology , where it shed new light on the analogies between bacterial heredity and the genetics of sexually @-@ reproducing organisms . French microbiologist André Boivin claimed to extend Avery 's bacterial transformation findings to *Escherichia coli* , although this could not be confirmed by other researchers . In 1946 , however , Joshua Lederberg and Edward Tatum demonstrated bacterial conjugation in *E. coli* and showed that genetics could apply to bacteria , even if Avery 's specific method of transformation was not general . Avery 's work also may have played a role in the continuation of X @-@ ray crystallography studies of DNA by Maurice Wilkins , who faced pressure from his funders to make whole cells , rather than biological molecules , the subject of his research .

Despite the significant number of citations to the paper and positive responses it received in the years following publication , Avery 's work was largely neglected by much of the scientific community . Although received positively by many scientists , the experiment did not seriously affect mainstream genetics research , in part because it made little difference for classical genetics experiments in which genes were defined by their behavior in breeding experiments rather than their chemical makeup . H. J. Muller , while interested , was focused more on physical rather than chemical studies of the gene , as were most of the members of the phage group . Avery 's work was also neglected by the Nobel Foundation , which later expressed public regret for failing to award Avery a Nobel Prize .

By the time of the 1952 Hershey ? Chase experiment , geneticists were more inclined to consider DNA as the genetic material , and Alfred Hershey was an influential member of the phage group . Erwin Chargaff had shown that the base composition of DNA varies by species (contrary to the tetranucleotide hypothesis) , and in 1952 Rollin Hotchkiss published his experimental evidence both confirming Chargaff 's work and demonstrating the absence of protein in Avery 's transforming principle . Furthermore , the field of bacterial genetics was quickly becoming established , and biologists were more inclined to think of heredity in the same terms for bacteria and higher organisms . After Hershey and Chase used radioactive isotopes to show that it was primarily DNA , rather than protein , that entered bacteria upon infection with bacteriophage , it was soon widely accepted that DNA was the material . Despite the much less precise experimental results (they found a not @-@ insignificant amount of protein entering the cells as well as DNA) , the Hershey ? Chase experiment was not subject to the same degree of challenge . Its influence was boosted by the growing network of the phage group and , the following year , by the publicity surrounding the DNA structure proposed by Watson and Crick (Watson was also a member of the phage group) . Only in retrospect , however , did either experiment definitively prove that DNA is the genetic material .