“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” (1944) by Oswald Avery, Colin MacLeod and Maclyn McCarty [1]

By: Hernandez, Victoria Keywords: bacterial transformation [2] transformation [3] DNA [4] Avery, Oswald [5] MacLeod, Colin [6] McCarty, Maclyn [7]

In 1944, Oswald Avery, Colin MacLeod, and Maclyn McCarty published an article in which they concluded that genes [8], or molecules that dictate how organisms develop, are made of deoxyribonucleic acid, or DNA. The article is titled “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,” hereafter “Transformation.” The authors isolated, purified, and characterized genes [8] within bacteria and found evidence that those genes [8] were made of DNA and not protein. Though scientists were initially skeptical that genes [8] were made of DNA, they later recognized that the data reported in “Transformation” were clear evidence that DNA was genetic material, a revelation that furthered research about how organisms grow, develop, and pass on traits to offspring.

In the early 1900s, many scientists supported the idea that genes [8] were made of protein. Scientists had verified that genes [8] were heritable, meaning that genes [8] could be passed from cell to cell, and from parent to offspring. They had also identified the different chemical constituents, or building blocks, of DNA and proteins. In 1933, Phoebus Levene, a researcher at The Rockefeller Institute [9] for Medical Research in New York City, New York, proposed that the building blocks of DNA had a regular, repeating sequence. Levene’s theory, called the tetranucleotide model, meant that the variability of DNA structure was limited. In contrast, scientists thought that the building blocks of protein could adopt many different structures. With those ideas, many researchers argued that genes [8] could not be made of DNA because DNA could not possess the necessary complexity to account for genetic variation, or the amount of genetic differences between individual organisms. When Avery, MacLeod, and McCarty wrote “Transformation,” their conclusion that genes [8] were made of DNA opposed many scientists’ hypotheses about the function of DNA.

To conduct the experiments detailed in “Transformation,” Avery, MacLeod, and McCarty used bacterial transformation. Bacterial transformation is the process by which bacteria can ingest and use new genetic material from its surroundings. In 1928, Frederick Griffith, a scientist at the British Ministry of Health in London, United Kingdom, reported that he observed bacterial transformation. He studied *Streptococcus pneumoniae* [10], also called pneumococcus, which is a species of bacteria that causes pneumonia, a lung disease in humans [11] and other animals. There are two types of pneumococcus, smooth and rough, which scientists label S and R, respectively. The living S form of pneumococcus causes disease, and the R form does not. When Griffith injected mice with a large amount of dead S form bacteria and a small amount of living R form bacteria, the mice still died. When Griffith analyzed the blood of those mice, he found living S form bacteria that continued to reproduce and make more S form bacteria. Griffith concluded that the dead S form bacteria must have exchanged some genetic material with the living R form bacteria, so the R form bacteria transformed into living S form. Griffith did not determine whether the living S form took up a genetic element, also called the transforming principle, made of protein or made of DNA. In the 1930s, Avery, MacLeod, and McCarty used and modified Griffith’s methods to answer their own questions.

Avery, MacLeod, and McCarty did not conduct all the experiments that they presented in “Transformation” together. In the early 1930s, Avery started researching bacterial transformation in pneumococcus to develop treatments for pneumonia. Around the same time, Avery received treatment for Graves’ disease, a disease that affects the thyroid gland. According to science historian Michael Fry, Avery experienced depression and tremors related to Graves’ disease and those symptoms limited his ability to work. MacLeod joined Avery’s lab in 1935 and they set out to identify the chemical nature of the transforming principle, but when

Avery could not work due to his illness, MacLeod worked alone. In 1941, MacLeod left Avery's lab and McCarty joined the lab to continue the bacterial transformation experiments with Avery. Avery, MacLeod, and McCarty worked on multiple projects in the 1930s and early 1940s, and by 1944, the scientists had enough data to publish "Transformation."

In "Transformation," a twenty-three-page article published in *The Journal of Experimental Medicine*, Avery, MacLeod, and McCarty describe their experiments on bacterial transformation and their results. "Transformation" has four major sections, which are "Experimental," "Discussion," "Summary," and "Conclusion." An introduction precedes the "Experimental" section. In the introduction, the authors describe prior experiments, including Griffith's. In the "Experimental" section, the authors detail their methods, from how they prepared the bacteria to how they purified and characterized their extracted transforming principle, the substance that caused bacterial transformation. Next, Avery, MacLeod, and McCarty provide the "Discussion" section in which they attempt to identify the chemical composition of the transforming principle. In the "Summary" section, the authors briefly review what they did in their experiments. Lastly, in the "Conclusion" section, the authors state their main finding identifying the transforming principle as DNA.

In the beginning of "Transformation," Avery, MacLeod, and McCarthy outline the context of their experiment. They explain that transformation in pneumococcus, or pneumonia-causing bacteria, is an example of how scientists could cause specific heritable changes in bacteria. The researchers discuss Griffith's experiment and other scientists' experiments that verified Griffith's results. One such experiment replicated Griffith's findings in vitro [12], meaning that the scientists demonstrated bacterial transformation in a test tube, as opposed to in vivo [13], or in living organisms like mice. Finally, Avery, MacLeod, and McCarty state that the purpose of their research is to identify the chemical nature of the transforming principle that causes transformation in bacteria.

The next section in "Transformation" is the "Experimental" section in which Avery, MacLeod, and McCarty detail the experiments they performed. The authors start the "Experimental" section by discussing the necessary conditions for inducing bacterial transformation in R form pneumococcus. That discussion includes details about the type of R form pneumococcus the researchers used and how they prepared that bacteria. Avery, MacLeod, and McCarty explain why they chose to use certain materials in their experiment and why they carried out experimental procedures in the ways that they did. Furthermore, Avery, MacLeod, and McCarty provide commentary on previous work conducted by other scientists, often attempting to explain other scientist's findings based on their own work.

Avery, MacLeod, and McCarty continue the "Experimental" section by describing how they quantified transformation activity in pneumococcus. In other words, the authors describe a method by which they could determine whether R form bacteria transformed into S form bacteria and the extent to which that transformation occurred. Without successfully inducing transformation in R form bacteria, the researchers would not be able to identify the transforming principle. As in the previous parts of the "Experimental" section, Avery, MacLeod, and McCarty include every quantity and every instrument used in their procedure. The researchers emphasize that their procedure preserves the material of interest, or the transforming principle. According to the authors, they prepared pneumococcus in a broth where R form and S form bacteria position themselves differently in the test tube. The authors state that in that broth, R form bacteria accumulates at the bottom of the test tube and leaves a clear liquid above. In contrast, the S form bacteria does not accumulate at the bottom, but instead disperses throughout the broth, making it cloudy. The authors conclude that they will know if transformation occurs when the clear liquid above the R form bacteria turns cloudy.

Next, the authors explain how they extract and purify the transforming principle. After they verify that transformation occurred, the researchers extract the heat-killed S form bacteria. They remove and purify the transforming principle from the bacteria so they can analyze it further. The authors document the temperature at which they conducted their experiment and the amount of time each step took. Avery, MacLeod, and McCarty state that the extracted transforming principle was a fibrous substance.

For the remainder of the "Experimental" section Avery, MacLeod, and McCarty discuss how they identified the transforming principle as DNA. First, they describe the physical and chemical properties of the extracted substance. They describe the effects that light, dissolving in water, and heating had on the transforming principle and its activity, or the extent to which bacteria transformed in the presence of the principle. In addition to observing the physical properties of the transforming principle, Avery, MacLeod, and McCarty describe how they performed various chemical tests. According to the authors, both the physical and chemical tests showed that the transforming principle was DNA, and not protein. For example, Avery, MacLeod, and McCarty state that they performed a test which revealed that the elements present in their sample were carbon, hydrogen, nitrogen, and phosphorus and how much of each element was present. According to the authors, the experimental ratios of those four elements supported the idea that the transforming principle was DNA, and not in protein. At this point in the "Experimental" section, the researchers tabulate their data.

Continuing the "Experimental" section, Avery, MacLeod, and McCarty explain how they used enzymes to characterize the transforming principle as DNA. Enzymes are molecules that facilitate chemical reactions in cells by interacting with the substances that participate in the chemical reaction. Enzymes are specific, meaning that a certain enzyme can only interact with certain substances. An enzyme that degrades protein cannot degrade DNA, and an enzyme that degrades DNA cannot degrade protein. The authors state that enzymes that degrade ribonucleic acid, or RNA, a molecular similar to DNA, and enzymes that degrade protein had no effect on the transforming principle. However, Avery, MacLeod, and McCarty write that when they exposed the transforming principle to an enzyme that degrades DNA, the enzyme broke down the transforming principle. The authors summarize their results of the enzyme tests in a table. The authors conclude that the results of their enzyme tests are further proof that the transforming principle is DNA.

Avery, MacLeod, and McCarty continue the “Experimental” section by describing additional tests they performed that provided evidence that the transforming principle was DNA. They state that the transforming principle sample did not contain any pneumococcal protein, the disease-causing substance of pneumococcus, and therefore the transforming principle must be structurally different from the pneumococcal protein. The researchers also state that they used an analytical ultracentrifuge, a centrifuge that spins substances rapidly and can separate different components in a mixture, to see whether the transforming principle was a mixture of different types of substances. After centrifugation, Avery, MacLeod, and McCarty found that no parts of the transforming principle separated. Instead, it was consistent throughout, which, according to the authors, meant that the transforming principle likely only contained one kind of substance. The authors identify that substance as DNA. Avery, MacLeod, and McCarty also write that the transforming principle absorbed the same type of light as DNA, an indication that the substances are likely the same. Avery, MacLeod, and McCarty conclude the “Experimental” section by describing the tests they used to characterize their transforming principle as DNA.

The following section in “Transformation” is the “Discussion” section. In that section, the authors interpret their experimental findings and conclude that the transforming principle was likely to be DNA. Avery, MacLeod, and McCarty state that, in their study, they successfully induced transformation in R form pneumococcus and that they extracted and purified DNA from the bacteria that caused R form bacteria to become S form bacteria. They explicitly state that the DNA sample, containing no protein, could induce transformation in bacteria. The researchers also note when the bacteria transformed, they generated a capsule, or a protective outer layer made of sugar molecules. The authors discuss that capsule generation seemed to be related to the transforming principle even though the capsule substance was made of different chemicals than the transforming principle. The authors do not discuss that any further in “Transformation.”

Next in the “Discussion,” Avery, MacLeod, and McCarty detail the reservations they have about claiming that the substance that facilitates transformation is DNA. The authors state that for a substance to induce transformation in bacteria, it must have a high degree of biological specificity. Biological specificity is the idea that certain characteristics of organisms, like behaviors or biochemicals, vary depending on the species. According to Avery, MacLeod, and McCarty, scientists had not explained how the DNA molecule, which had little structural variability when compared with proteins, could allow for biological specificity. For that reason, the authors do not claim that DNA is indisputably the cause of bacterial transformation. Avery, MacLeod, and McCarty end their “Discussion” by proposing that transformation in bacteria could be due to small amounts of undetectable impurities also present in their experimental DNA sample. However, the researchers state that if other experiments support their findings, DNA must facilitate transformation in bacteria. After the “Discussion” section, Avery, MacLeod, and McCarty end their paper with the “Summary,” “Conclusion,” and “Bibliography” sections.

“Transformation” did not fully convince scientists that genetic material was made of DNA in the 1940s. Many other scientists at the Rockefeller Institute [9] for Medical Research, where Avery, MacLeod, and McCarty worked, were skeptical of the findings in “Transformation”. In their paper, Avery, MacLeod, and McCarty did not completely rule out the possibility of some substance other than DNA causing the genetic changes required for bacterial transformation. Historians have noted that, privately, Avery was more confident that DNA facilitated bacterial transformation.

Many scientists disputed the findings in “Transformation.” When “Transformation” was published, the Phage Group, an organization [14] of scientists who studied bacteriophages, or viruses that infect bacteria, expressed doubt about Avery, MacLeod, and McCarty’s conclusions. Max Delbrück, one of the founders of the Phage Group, stated that scientists resisted the idea that DNA was genetic material because they thought DNA was too simple to be genetic material. According to historians, Avery, MacLeod, and McCarty challenged scientists’ entire understanding of what DNA and genes [8] were and so the scientific community largely resisted the claims they made.

Many scientists did not accept Avery, MacLeod, and McCarty’s conclusions in “Transformation” until more researchers conducted experiments that supported the idea that the transforming principle was DNA. In 1952, almost a decade after “Transformation,” Alfred Hershey and Martha Chase, two researchers at the Carnegie Institution of Washington [15] in Cold Spring Harbor, New York, verified Avery, MacLeod, and McCarty’s findings. In what scientists later called the Hershey-Chase experiments, Hershey and Chase demonstrated that bacteriophages injected DNA into bacteria during infection, and that the phage DNA was the genetic element that replicated and created new bacteriophage particles inside the bacteria. Unlike Avery, MacLeod, and McCarty’s results, scientists widely accepted the article published about the Hershey-Chase Experiments. Historians have discussed why scientists were more receptive to the conclusions of the Hershey-Chase Experiments and not to those of "Transformation." Some historians argue that the authors published "Transformation" during a time when scientists rarely considered that genes [8] could be made of DNA rather than protein, whereas the Hershey-Chase experiments occurred after scientists had more exposure to that idea. A year after the Hershey-Chase experiments, in 1953, James Watson [16] and Francis Crick [17], two scientists at the University of Cambridge in Cambridge, United Kingdom, modeled the structure of DNA in such a way that demonstrated how DNA could provide biological specificity as genetic material, thereby supporting Hershey and Chase's and Avery, MacLeod, and McCarty's findings. Years after scientists determined that DNA was genetic material, settled the structure of DNA, and how DNA functioned, scientists considered the experiments and data Avery, MacLeod, and McCarty discussed in "Transformation" to be some of the earliest evidence that genes [8] were made of DNA.

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Subject

Transformation, Bacterial [25] Bacterial transformation [26] Streptococcus pneumoniae [27] Avery, Oswald Theodore, 1877-1955 [28] MacLeod, Colin M. (Colin Munro), 1909-1972 [29] McCarty, Maclyn [30] Bacterial genetics [31] Transformation (Genetics) [32] Desoxyribonucleic acid [33] Griffith, F. (Frederick), 1879-1941 [34] Deoxyribonucleic Acid [35] Genetics [36]

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