

# The Hershey-Chase Experiments (1952), by Alfred Hershey and Martha Chase

In 1951 and 1952, Alfred Hershey and Martha Chase conducted a series of experiments at the Carnegie Institute of Washington in Cold Spring Harbor, New York, that verified genes were made of deoxyribonucleic acid, or DNA. Hershey and Chase performed their experiments, later named the Hershey-Chase experiments, on viruses that infect bacteria, also called bacteriophages. The experiments followed decades of scientists' skepticism about whether genetic material was composed of protein or DNA. The most well-known Hershey-Chase experiment, called the Waring Blender experiment, provided concrete evidence that genes were made of DNA. The Hershey-Chase experiments settled the long-standing debate about the composition of genes, thereby allowing scientists to investigate the molecular mechanisms by which genes function in organisms.

In the early twentieth century, scientists debated whether genes were made of DNA or protein. Genes control how organisms grow and develop and are the material basis for organisms' ability to inherit traits like eye color or fur color from their parents. By 1900, scientists had identified the complete chemical composition, or building blocks, of DNA. They had also verified that all cells contained DNA, though DNA's function remained ambiguous. Up until the 1940s, some scientists accepted the idea that genes were not made of DNA. Instead, those scientists supported the idea that DNA was a molecule that maintained cell structure. Scientists supported that idea in part because of a hypothesis called the tetranucleotide hypothesis. Phoebus Levene, a researcher at the Rockefeller Institute for Medical Research in New York City, New York, proposed the tetranucleotide hypothesis for DNA in 1933. According to Levene and other proponents of the hypothesis, DNA consisted of repeating sets of four different building blocks, called nucleotides. Some scientists concluded that a repeating sequence of nucleotides in DNA limited potential for variability. Those scientists considered variability necessary for DNA to function as genetic material. In other words, genes needed to have the capacity for enough variation to account for the different traits scientists observe in organisms. Conversely, scientists found that proteins had many more building blocks and therefore more possible arrangements than DNA. From that, some scientists claimed that genes must have been made of protein, not DNA.

The Hershey-Chase experiments were not the first studies to oppose the prevailing theory in the early 1900s that genetic material was composed of proteins. In 1944, nearly a decade before Hershey and Chase's work, scientists published sound evidence that genes were made of DNA rather than protein. Starting in 1935, Oswald Avery, another researcher at the Rockefeller Institute, with his research associates Colin MacLeod and Maclyn McCarty, performed experiments that showed that DNA facilitated a genetic phenomenon in bacteria called bacterial transformation. Bacterial transformation is the process by which a bacterium can get and use new genetic material from its surroundings. During bacterial transformation, a non-disease-causing bacterium can transform into disease-causing bacteria if the non-disease-causing bacteria is exposed to a disease-causing bacteria. Transformation can occur even if the disease-causing-strain is dead, implying that bacterial transformation happens when the non-disease-causing bacteria inherits genetic material from the disease-causing bacteria. Avery and his colleagues found that the inherited factor that caused bacterial transformation contained DNA. However, Avery's group did not discount the possibility that some non-DNA component in their sample caused bacterial transformation, rather than the DNA itself. Because of that, many scientists maintained the idea that proteins must govern the genetic phenomenon of bacterial transformation.

Starting in 1951, Alfred Hershey and Martha Chase conducted a series of experiments, later called

the Hershey-Chase experiments, that verified the findings of Avery and his colleagues. Hershey was a researcher who studied viruses at the Carnegie Institution of Washington in Cold Spring Harbor, New York. He studied viruses that infect bacteria, also called bacteriophages, or phages. Chase became Hershey's research technician in 1950.

In their experiments, Hershey and Chase analyzed what happened when phages infect bacteria. By the 1950s, scientists had evidence for how phages infected bacteria. They found that when phages infect a host bacterium, the phages first attach themselves to the outside of the bacterium. Then, a piece of the phage enters the bacterium and subsequently replicates itself inside the cell. After many replications, the phage causes the bacterium to lyse, or burst, thereby killing the host bacteria. Scientists classified the replicating piece as genetic material. Scientists also found that phages contained two classes of biological molecules: DNA and protein. Hershey and Chase sought to determine if the replicating piece of phages that entered bacteria during infection, the genetic parts, were solely DNA.

To perform their experiments, Hershey and Chase utilized a technique called radioactive isotope labeling. Chemical elements can exist in different structural forms called isotopes. Isotopes of the same element are nearly identical, but scientists can distinguish between them by experimental means. One way to differentiate between chemical elements with different isotopes is by analyzing their radiation. Some isotopes are less stable than others and give off radioactive signals that scientists can detect. Hershey and Chase marked phages by incorporating radioactive isotopes of phosphorus and sulfur in those phages. They allowed some phages to replicate by infecting bacteria, specifically *Escherichia coli*, or *E. coli*, that scientists had grown in radioactive sulfur. The researchers let other phages infect and replicate in *E. coli* that scientists had grown in radioactive phosphorus. DNA contains phosphorus, but not sulfur, whereas protein contains sulfur, but not phosphorus. Therefore, when Hershey and Chase marked phages with radioactive isotopes of those elements, they placed separate, distinguishable tags on the protein and DNA parts of the phages.

The first Hershey-Chase experiment aimed to confirm previous experimental findings that the DNA and protein components of phages were separable. In 1950, Thomas Anderson at the University of Pennsylvania in Philadelphia, Pennsylvania, showed that phages consisted of a protein shell, or coat, with DNA inside the shell. Anderson found that the phages could release their DNA and leave behind what he called a protein ghost. Hershey and Chase replicated Anderson's experimental results using their radioactive isotope labeling method. Hershey and Chase were able to separate the phages into radioactive sulfur-containing protein ghosts and radioactive phosphorus-containing DNA. They found that the radioactive sulfur protein ghosts could attach to bacterial membranes while the radioactive phosphorus DNA could not. Hershey and Chase also tested if enzymes, molecules that facilitate chemical reactions in cells, could degrade DNA. They found that enzymes did not degrade the DNA of intact phages, but did degrade the DNA of separated phages. Those results indicated that in the intact phages, the protein coat surrounded the DNA and protected the DNA from degradation.

In another Hershey-Chase experiment, Hershey and Chase showed that when certain phages infected *E. coli*, the phages injected their DNA into the host bacterium. In 1951, Roger Herriot at Johns Hopkins University in Baltimore, Maryland, demonstrated that after phages infected bacteria, their protein ghosts remained attached to the outside of the bacterial cells while their DNA was released elsewhere. Hershey and Chase aimed to show where the phage DNA went when it exited the protein coat and entered the bacteria. The researchers allowed radioactive phosphorus-labeled phages to attach to bacterial cell membranes in a liquid solution and infect the bacteria. Using a centrifuge, Hershey and Chase rapidly spun the samples to separate the bacterial cells from the surrounding solution. After centrifugation, they found that most of the radioactive phosphorus was detected in the cells rather than in the surrounding solution, meaning that the phage DNA must have entered the cells when the phages infected the bacteria.

The most well-known Hershey-Chase experiment was the final experiment, also called the Waring Blender experiment, through which Hershey and Chase showed that phages only injected their DNA into host bacteria, and that the DNA served as the replicating genetic element of phages. In the previous experiment, Hershey and Chase found evidence that phages injected their DNA into host bacteria. In the Waring Blender experiment, the scientists found that the phages did not inject any

part of their protein coats in the host bacteria and the protein coats remained outside the bacteria, adhered to the bacterial membranes. For their experiment, Hershey and Chase prepared two samples of infected *E. Coli*. They infected one sample with radioactive phosphorus-labeled phages, and the other sample with radioactive sulfur-labeled phages. Then, they stirred each sample in a Waring Blender, which was a conventional kitchen blender. They used a blender because centrifuges spun too fast and would destroy the bacterial cells. The shearing forces of the blender removed the phage particles that adhered to the bacterial membranes, but preserved the integrity of the cells and most of the phage material that entered the cell. In the phosphorus-labeled sample that marked DNA but not protein, the blender removed forty percent of the labeled particles. In the sulfur-labeled sample that marked protein but not DNA, the blender removed eighty percent of the labeled particles. Those results indicated that the blender removed much more of the protein parts of the phage than the DNA parts, suggesting that the protein likely remained adhered to the outside of the cell during infection. Since the protein remained outside the cell, it could not be the replicating genetic material.

The Waring Blender only removed eighty percent of the radioactive sulfur-labeled phage, so Hershey and Chase could not account for twenty percent of the phage protein material. To show that the missing twenty percent of the phage protein did not enter the bacterial cells and replicate, the researchers infected *E. Coli* with radioactive sulfur-labeled phage again so that only the protein parts of the phage were labeled. They prepared two samples. For one sample, Hershey and Chase stirred the cells in the blender to remove the phage particles adhered to the outer bacterial membrane. After stirring, they allowed the phages to cause the cells to lyse, releasing newly replicated phages. For the second sample, Hershey and Chase did not stir the cells in the blender and measured the resulting replicated phages after the bacterial cells lysed. In the blender-stirred sample, less than one percent of the replicated phages contained the radioactive sulfur label. However, in the sample that Hershey and Chase did not stir in the blender, almost ten percent of the phages contained the radioactive sulfur label. The blender maintains any phage material that entered the bacterial cell. If protein was genetic material that entered the cell and replicated, then Hershey and Chase would have found more sulfur-labeled protein in the sample they stirred with the blender. The sample that they did not stir had more of the sulfur-labeled protein because the protein coats remained on the outside of the cell. Hershey and Chase concluded that protein was not genetic material, and that DNA was genetic material.

Unlike Avery's experiments on bacterial transformations, the Hershey-Chase experiments were more widely and immediately accepted among scientists. The Hershey-Chase experiments mostly ended scientists' suspicions that genes were made of protein rather than DNA. However, historians have questioned the conclusiveness of the Hershey-Chase experiments. In all the Waring Blender experiments, some protein and DNA material remained unaccounted for. Even in the final experiment, when Hershey and Chase allowed the bacterial cells to lyse after stirring in the blender, the scientists still recovered a small amount of protein, implying that some protein entered the cells during infection. Furthermore, the amount of contaminating protein in the Hershey-Chase Experiments exceeded the amount of contaminating protein that Avery's group found in their experiments.

Historians of science have studied why scientists more readily accepted the Hershey-Chase experiments than Avery's experiments. Science historian Frederic Lawrence Holmes writes that scientists more readily accepted the results of the Hershey-Chase experiments because Hershey communicated directly with skeptical scientists. Hershey sent letters to his colleagues in which he detailed the experimental findings of the Hershey-Chase experiments. Another historian of science, Michel Morange, writes that the Hershey-Chase experiments were performed at a time when scientists were ready to accept that genetic material could be DNA. Avery's group conducted their experiments when the tetranucleotide hypothesis was popular and few scientists held that genes contained DNA. According to Morange, because Hershey and Chase conducted their experiments years later, scientists had gathered more experimental evidence and were willing to seriously consider that genes contained DNA.

In 1953, James Watson and Francis Crick, two scientists at the University of Cambridge in Cambridge, England, modeled the three-dimensional structure of DNA and demonstrated how DNA might function as genetic material. In 1969, Hershey shared the Nobel Prize in Physiology or

Medicine with two other scientists, Max Delbrück and Salvador Luria, partly for his work on the Hershey-Chase experiments.

## Sources

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