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WHO WOULD HAVE GUESSED IT?

The greatest single achievement of nature to date was surely the invention of the molecule DNA.

—LEWIS THOMAS, *THE MEDUSA AND THE SNAIL*

THE PLANES WERE COMING again.

After a respite of more than a month, Londoners were once more taking cover at the wail of air raid sirens, and listening anxiously for the drone of approaching aircraft. More than 60 times since September 7, 1940, the first night of the Blitz when over 250 German bombers pounded the capital with high explosives, bombs, and incendiaries, residents had braced themselves against the din of antiaircraft guns, the whistling of falling bombs, and the stomach-churning roar of explosions and collapsing buildings.

In the first eight months of Germany's campaign to bomb Britain into submission, more than 20,000 civilians were killed in London alone. Hundreds of thousands of buildings were destroyed or damaged, and more than 1 million people lost their homes. Countless landmarks, including Buckingham Palace and the Houses of Parliament, were badly damaged. Shipping and transportation were crippled—food and fuel were scarce and rationed.

But somehow London and Britain had endured. In a national radio address, Prime Minister Winston Churchill praised the British people for standing up against Hitler's "blackmail by murder and terrorism." He lauded the police, fire, ambulance, and rescue workers who had responded to the country's ordeal with tremendous bravery. Churchill also praised the medical and public health

authorities who had managed to prevent any increase in illness, despite the crowded conditions in air raid shelters and the disruptions to water and sewage lines.

One of those authorities was Dr. Frederick Griffith, a 62-year-old microbiologist who helped set up the Emergency Public Health Laboratory at the outset of the war. A staunch patriot, Griffith also contributed his aluminum pots and pans to help build Spitfires, bought war bonds, and steadfastly refused to move out of London "for any German."

With all citizens doing their part, Churchill reassured his listeners, "We shall not fail or falter; we shall not weaken or tire."

But this night, April 16, 1941, would be one of London's worse nights and greatest tests. The attack would be much larger, one of the heaviest since the war began. Beginning around nine o'clock in the evening and lasting until nearly dawn, 685 bombers would discharge their payloads over the city. In addition to the 890 tons of high explosives and 150,000 incendiaries, the Germans dropped parachute mines, large naval mines attached to parachutes that would descend slowly and land on building rooftops, then detonate seconds later. The weapon had a much larger blast area than bombs that detonated on impact, and had proven particularly effective at maximizing destruction in cities.

Just after midnight, a parachute mine landed on Griffith's house at 75 Eccleston Square in the historic and handsome borough of Westminster. The ensuing blast leveled the building and damaged several nearby structures. Ambulances and rescue parties were sent to the area. They worked through the night and into the morning to search the rubble for survivors and victims. The frantic scene was repeated at hundreds of locations across the city.

Griffith did not survive; he was one of more than 1000 Londoners who perished in the raid. A soft-spoken, reclusive man who was unmarried, had no children, and few close friends, Griffith was not the sort of personality to be long remembered by posterity. But years earlier, he had made an astonishing discovery that would lead to the surprising revelation that DNA was the chemical basis of heredity, and ensure that his legacy in the story of genetics would endure.

ADVENTURES WITH PNEUMONIA

Griffith had devoted his life to studying the bacteria that caused diseases, including tuberculosis, pneumonia, and scarlet fever. He firmly believed that progress in treating infectious diseases and preventing epidemics would require

precise knowledge of the microbes responsible. One of the first requirements was to be able to identify and classify different species of bacteria, or different strains of one species involved in infections.

Much of Griffith's focus had been shaped by war. At the end of his life, he was studying bacteria isolated from war wounds. Twenty years earlier, following the end of the First World War, he focused on bacteria that played a part in a deadly worldwide pandemic. Toward the end of that war (1918), an influenza pandemic erupted that eventually killed 20 million to 50 million people, many more than died in combat. For most victims, it was not the flu virus itself that was lethal, but secondary infections that followed. One of the most common culprits was the bacterium *Streptococcus pneumoniae*, a normal inhabitant of nasal and lung linings. Griffith was keen to understand how and why this "ubiquitous and apparently harmless organism may suddenly become more pathogenic . . . and propagate an epidemic."

It was known that there were several types of *S. pneumoniae*. Types I, II, and III were each distinguished by antibodies from people or animals that had been exposed to the bacteria and reacted specifically with chemical structures on the gelatinous capsule that coated the bacteria. A fourth heterogeneous group was called Type or Group IV. Griffith discovered that *S. pneumoniae* were even more diverse than these four types. When cultured on a bacterial plate, colonies of the four major types have a smooth, rounded, and glistening appearance due to their capsules. Griffith discovered another variant in which colonies were small with irregular, rough edges, and lacked capsules (Figure 4.1). When bacteria were injected into mice, Griffith observed that all of the "smooth" or S forms were virulent (caused disease) and fatal, whereas the "rough" or R form was not ("avirulent").

From mice inoculated with samples from one advanced infection, however, Griffith obtained an unexpected mixture of both smooth and rough colonies of bacteria that grew up on an agar plate. When reinoculated into mice, three colonies of the rough forms were avirulent, as expected, but one rough colony caused a fatal infection. This was the first time Griffith or anyone else had observed a virulent rough strain. The rough colony belonged to Type IV.

Griffith decided to investigate this unusual microbe. Since previous rough forms were derived from cultures of smooth bacteria, he wanted to see whether he might be able to revert the rough form back into a fully smooth form. After a long series of passages in mice and culture in the laboratory, he obtained a small shiny colony similar to the smooth type that was still virulent. He then tried to repeat the feat with a rough, avirulent Type II strain. Griffith found

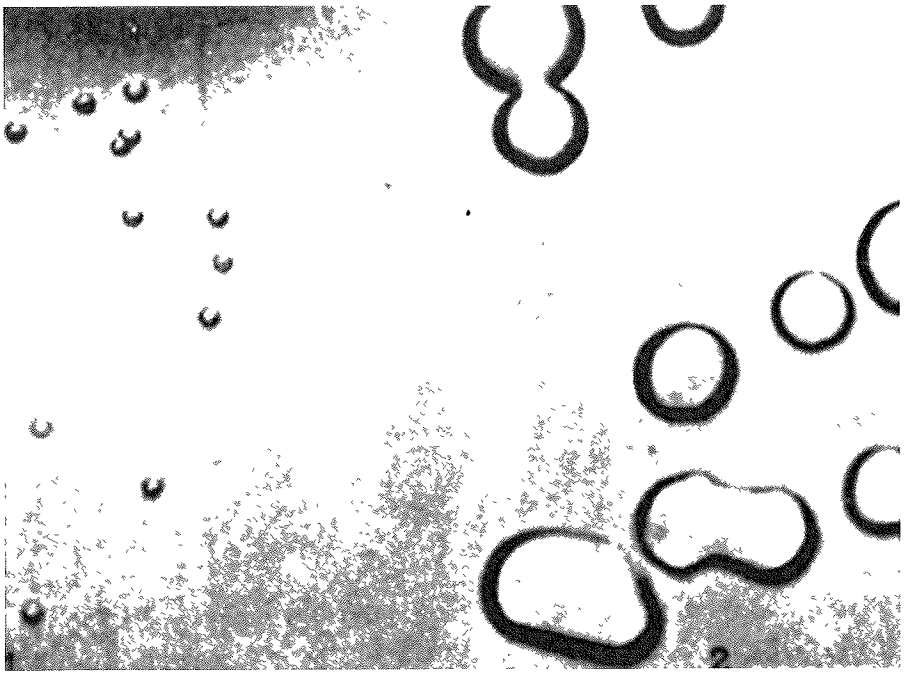

FIGURE 4.1 Smooth and Rough Forms of *S. pneumoniae* The smooth, virulent forms appear so because of a polysaccharide capsule around the bacterium; rough forms lack the capsule and form smaller colonies. Image courtesy of Rockefeller Archive Center

that by starting with a large dose of bacteria in mice, he was able to obtain a smooth, virulent form.

How this change in type occurred was a mystery. The latter result gave Griffith the notion that something about the large dose of bacteria was important for transforming a strain from the rough avirulent form to a smooth virulent form. The structures on the capsular surface of the bacteria that determined their types were known to be complex carbohydrates. Rough bacteria lacked a capsule and those carbohydrates, but Griffith wondered whether there might be some trace of a carbohydrate-synthesizing enzyme that remained, such that when a large number of rough cells was congregated, some might have their type-specific carbohydrate restored.

Thinking that such an enzyme might be vulnerable to heat, he started testing whether bacteria killed by heating at various temperatures might also be able to transform a small quantity of live avirulent bacteria into virulent bacteria. The experiment worked—the combination of killed bacteria and

live, avirulent rough bacteria injected into mice produced smooth, virulent colonies. Control experiments demonstrated that neither preparation alone (killed bacteria or live avirulent bacteria) was sufficient to produce any transformation.

In his initial experiments with killed cells, Griffith transformed a rough strain with killed preparations of the same type. He expected that this striking effect would be limited to combinations of cells of the same type. Griffith then tried mixing rough avirulent strains derived from one type with killed preparations of another type and injected the combination into mice. To his surprise, he obtained smooth, virulent strains—all of which were of the killed bacteria type!

The result was so unexpected that Griffith was careful to be absolutely certain that the killed preparation contained no live bacteria that would undermine the experiment. It did not. Griffith repeated the experiment with various combinations of killed and live types (Figure 4.2). The rough strains were transformed to the killed bacteria type.

Griffith wasn't the only one who was surprised. His report of the transformations of *S. pneumoniae* types stunned fellow microbiologists. Oswald Avery, for example, an eminent American *Streptococcus* expert, believed that bacterial types were stable. He found it hard to accept that *Streptococcus* could change from one type into another. That was until someone in his own lab repeated Griffith's results while Avery was away on summer vacation. Griffith had earned the reputation for being very careful, and he described his experimental methods in such exact detail that other laboratories were also able to promptly confirm his findings.

But just what was the substance responsible for the dramatic transformation of types? What sort of chemical could induce a heritable change in bacteria? The identity of the transforming substance was still unknown at the time of Griffith's death 13 years after his discovery. Griffith himself did not pursue the question—he was more concerned with the medical and epidemiological significance of his findings. But across the Atlantic, far from the nightmare in London, one group had picked up the trail, and was getting close to an answer that would stun the scientific world.

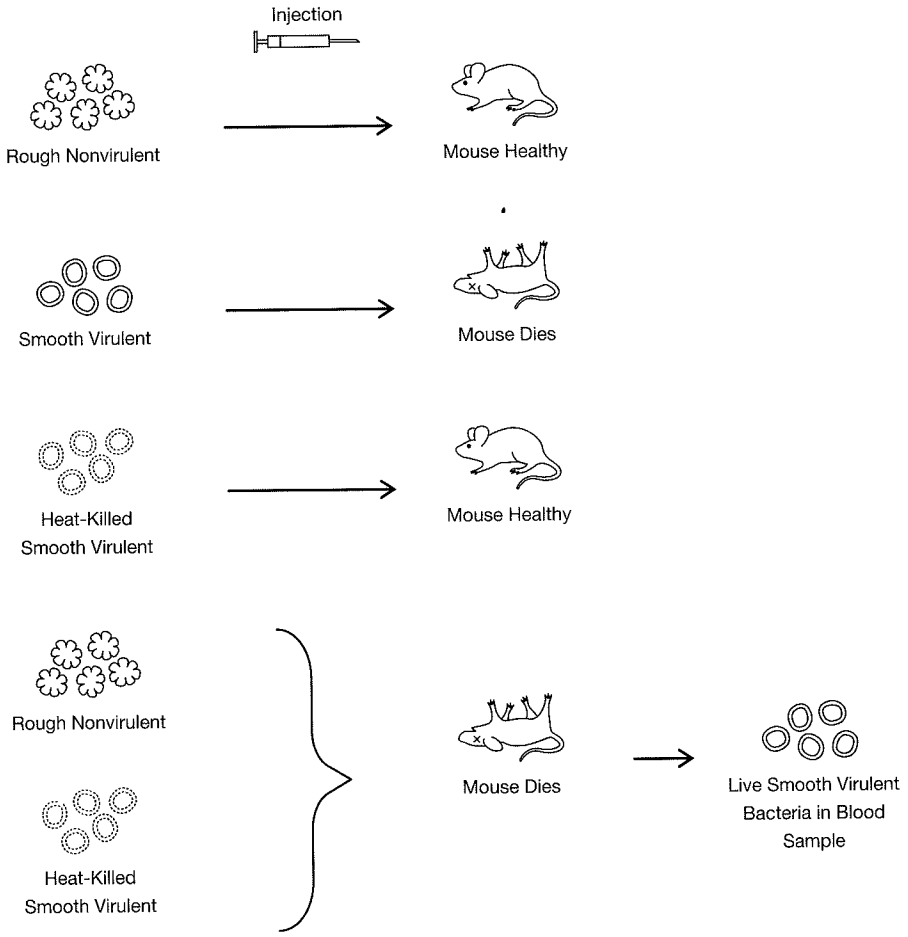

FIGURE 4.2 Griffith's Discovery of the Transformation of Bacterial Types Schematic of Griffith's experiment in which he injected rough or smooth strains, a heat-killed smooth strain, or a mixture of a live rough and a heat-killed smooth strain. The unexpected result was the lethality of the mixture, and his recovery of a live virulent, smooth strain of the same type as the killed strain.

FESS

Oswald Avery and Fred Griffith had two important traits in common—both were trained physicians with a passion for understanding *Streptococcus* pneumonia, and both had a very cautious scientific nature. Each man was determined to understand and to help thwart the disease, and careful not to overstate

FIGURE 4.3 Oswald T. "Fess" Avery

Science History Images/Alamy Stock Photo

any implications of their own work. But in other ways, the two men were opposites. Whereas the painfully shy Griffith avoided social situations, Avery was welcoming to any visitor or stranger, and far more outgoing and comfortable in conversation. His warmth attracted talented junior scientists to work in his group, and he was gifted with such impressive verbal skills that students and his colleagues at Rockefeller University Hospital in New York affectionately referred to him as the Professor or Fess (Figure 4.3).

Avery initially had strong doubts about the transformation of *Streptococcus* types, but once he was convinced the phenomenon was real, he started to

chase after the transforming substance. The experiments were very difficult, and there were other, more urgent priorities, so the work progressed in fits and starts, with some long dormant spells. In October 1940, Avery and Colin MacLeod, a young Canadian physician, together resumed the hunt.

Over the previous decade, Avery's group had made two important advances. First, they had succeeded in transforming bacteria in laboratory culture, thereby eliminating the time-consuming process and variable outcomes of inoculating live mice. And second, they were able to produce an extract from bacteria that replicated the transforming activity of killed bacteria. With a simpler test for activity, and an active extract, they could potentially purify and identify the transforming substance (or what Avery called the transforming "principle").

The thick, syrupy extracts contained all of the classes of large molecules in bacterial cells: proteins, lipids, carbohydrates, and nucleic acids. That complexity was not the only challenge to purification—the transforming activity itself was destroyed by enzymes present in the extracts. In 1940, there were very few established techniques for purifying or analyzing large molecules. One good rule of thumb for any purification strategy was to be sure to start with as much active material as possible. Despite efforts to make extracts in the same way, however, their activity was highly variable, and sometimes zero. "Disappointment is my daily bread," Avery would often say.

MacLeod and Avery focused on trying a number of techniques to separate the extracts into different classes of molecules. What unfolded was essentially a process of elimination in which different classes of molecules were removed one by one from the extracts. In the winter of 1940–41, MacLeod and Avery learned that the chemical chloroform ($CHCl_3$) was a very effective way of removing protein (and destructive enzymes) from the extract. They also found out that adding ribonuclease enzyme to destroy ribonucleic acid (RNA) had no effect on activity. Once they figured out how to obtain more active material from bacteria, they scaled up their preparations from 2- or 3-liter cultures of cells to 50-liter batches.

One of the separation steps involved precipitation of the transforming activity with alcohol. This precipitate contained a large amount of the capsular polysaccharide, which raised the possibility that it played some role in transformation. MacLeod had to leave the lab for other duties and Maclyn McCarty, another young physician, joined Avery's lab. McCarty assessed the role of the polysaccharide by destroying it with a specific enzyme. He found that the transforming activity was unaffected. The transforming activity was made of something else.

McCarty was able to make extracts that were essentially devoid of proteins or polysaccharides. What substances remained in the extract? In chemical tests,

the extract was positive for the sugar deoxyribose. This was an indicator of the presence of deoxyribonucleic acid (DNA), but it did not prove that the transforming principle was made of DNA because other substances were also present. McCarty noticed that DNA had the same fibrous and viscous appearance as his preparations of transforming principle. So McCarty decided to test the ability of various crude enzyme preparations that broke down (depolymerized) DNA to affect transforming activity. He found a perfect correlation between the depolymerization of DNA and the destruction of transforming activity.

Still, these results were only suggestive that DNA *could* be the substance responsible. McCarty and Avery knew the stakes were too high to get it wrong. They had to eliminate the possibility that some other contaminating substance carried the activity. What they needed was as pure a preparation of bacterial DNA as they could get. McCarty figured out how to fractionate and purify DNA and he found that essentially all of the transforming activity resided in his DNA fraction.

Up to this time, biologists knew nothing about the function of DNA, not even whether it was present in most species. And chemists knew only that DNA was composed of just four nucleotides—its structure was unknown. Proteins, made of long chains of 20 different amino acids, were believed to be responsible for specific biological activities within cells.

In May 1943, Avery wrote to his brother Roy, also a scientist, to share the news:

> For the past two years, first with MacLeod and now with Dr. McCarty I have been trying to find out what is the chemical nature of the substance which induces this specific change. . . . Some job, full of headaches and heartbreaks. But at last perhaps we have it. . . .
>
> In short, this substance is highly reactive and on elementary analysis conforms very closely to the theoretical values of pure desoxyribose nucleic acid. . . . (Who could have guessed it)

The work had not yet been submitted for publication or reviewed, so Avery maintained a mixture of caution and excitement:

> If we are right and of course that is not yet proven, then it means that . . . by means of a known chemical substance it is possible to induce predictable and hereditary changes in cells. This is something that has long been the dream of geneticists.

NO NOBEL?

The discovery was published in February 1944, when the world and much of the scientific community was engrossed in the war. It took a while for biologists to become aware of the report. Reactions were mixed. Some found it difficult to believe that DNA, which was thought to be a polymer made up of four repeating bases (ACGTACGTACGT) could carry specific information. Some thought the transforming activity might be a phenomenon peculiar to bacteria. Others, however, thought it was the most exciting breakthrough in all of biology.

It would take several years and further discoveries by others to convince the skeptics, and to shed light on just how DNA could carry information. Avery, who turned 67 in 1944, had put off retiring to pursue the transforming substance. He fully retired in 1948 and died in 1955, two years after Watson and Crick deciphered the structure of DNA (Chapter 5).

One might think that the discovery of the function of DNA would certainly have earned Avery the Nobel Prize. But Nobels are not awarded posthumously, and the Nobel Committee did not recognize the significance of Avery's work in time. The chairman of the committee later admitted that overlooking Avery was one of the Nobel's greatest oversights. Of course, Avery's discovery had only been made possible by Griffith's original, difficult, and surprising experiments. Griffith, too, was ineligible, and also never recognized.

END-OF-CHAPTER QUESTIONS

1. What was Fred Griffith's very surprising observation that eventually led to the discovery of the transforming principle and DNA as the hereditary material?
2. What early advances enabled Avery's group to pursue the eventual purification of the transforming principle?
3. The following table is from Avery, MacLeod, and McCarty's paper on the transforming principle. It shows which crude enzyme preparations contained certain activities and which were able to inactivate the transforming principle.