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Bacterial Transformation and the Origins of Epidemics in the Interwar Period: The Epidemiological Significance of Fred Griffith's "Transforming Experiment"*

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Abstract. Frederick Griffith (1879–1941) was an English bacteriologist at the Pathological Laboratory of the Ministry of Health in London who believed that progress in the epidemiology and control of infectious diseases would come only with more precise knowledge of the identity of the causative microorganisms. Over the years, Griffith developed and expanded a serological technique for identifying pathogenic microorganisms, which allowed the tracing of the sources of infectious disease outbreaks: slide agglutination. Yet Griffith is not remembered for his contributions to the biology and epidemiology of infectious diseases so much as for discovering the phenomenon known as 'transformation'. Griffith's discovery, for many, was a pure case of serendipity whose biological relevance had also largely escaped him. In this paper, I argue that the key to understanding the significance of bacterial transformation – and the scientific legacy of Fred Griffith – rests not only on it initiating a cascade of events leading to molecular genetics but also on its implications for epidemiology based on the biology of host–parasite interactions. Looking at Griffith's entire career, instead of focusing only on the transformation study, we can better appreciate the place of the latter within Griffith's overall contributions. Presented in this way, Griffith's experiment on bacterial transformation also ceases to appear as an anomaly, which in turn leads us to rethink some of the most prevalent historical conceptions about his work.

* This paper is dedicated to the memory of Dr. Richard M. Krause (1925–2015), director of the National Institute of Allergy and Infectious Diseases from 1975 to 1984, "amateur" historian of science (in the original sense of the word), and philanthropist.

Keywords: Epidemics, Serological transformation, Host–parasite interactions, Virulence, Bacteriology, Evolution, Oswald Avery, Rebecca Lancefield, Joshua Lederberg

Introduction

"Mutation of type among disease-producing bacteria" was, according to Frederick Griffith (1879–1941), "a subject of obvious importance in the study of epidemiological problems" (1928, p. 154). Working as a public health officer at the Pathological Laboratory of the Ministry of Health in London, Griffith was an English bacteriologist who strongly believed that progress in the epidemiology of infectious diseases would come only with more precise knowledge of the identity of the causative microorganisms.[1] A better understanding of the ways bacterial strains undergo changes and variations, Griffith thought, was a precondition of a better control of infectious diseases through the production of specific vaccines and curative antisera. It was accordingly important to assess the level of variability of specific bacterial strains. To this end, he devoted three decades of his professional life to the classification of pathogenic bacterial species, producing a systematic grouping of these microorganisms through the use of an identification technique he had developed and extended over the years: slide agglutination.[2]

From 1901 to 1941, Griffith investigated various disease outbreaks across Britain and sought to identify the causative agents. His early

[1] As medical microbiologist A.W. Downie emphasized in the Fourth Griffith Memorial Lecture, "Griffith's main scientific interests were related to the epidemiology of infectious disease" (1972, p. 2). Historian of science Robert Olby pointed out, similarly, that for Griffith "[t]o learn how to control the incidence and spread of chronic lobar pneumonia called for a detailed knowledge of the host–bacterium relationship" (1994, p. 178). See also Wright (1941).

[2] Bacterial strains of a same species usually display a wide array of antigenic differences expressed on the cell surface structures. To determine the type of strain under study, Griffith used what is called the slide agglutination technique. This test proceeds thus: the serum derived from infected animals (often a mouse, a guinea-pig, or a horse) is cultivated and mixed with the type of cell used to cause the infection. Then, as the antigen–antibody relation is specific, if the two agglutinate in the sera this means that antibodies are binding with corresponding antigens in the bacterial strain. Using this procedure, researchers could determine the identity of the strain they were dealing with and could produce therapeutic serum to cure a patient or to induce immunity. This method allowed for practical innovations, such as the identification of pathogenic and non-pathogenic strains, and made possible the epidemiological study of infectious diseases caused by microorganisms (see Dubos, 1976, p. 133).

major work focused on the relation between bovine and human tuberculosis, a research conducted together with his older brother, Arthur Stanley Griffith (1875–1941), as part of the ten-year long investigation of the Royal Commission on Tuberculosis (on the Commission, see Francis, 1959). During the following decades at the Pathological Laboratory at the Ministry of Health, Griffith reported on several aspects of the biology and epidemiology of meningococcus, pneumococcus, and streptococcus. He also developed a new test for detecting syphilitic infections. Griffith's work on the classification of hemolytic streptococcus is especially important from a public health point of view as the typing of bacteriological strains permitted the identification of the sources of various acute infections such as rheumatic fever, scarlet fever, puerperal fever, and other minor wound infections such as sore throats (see Hare, 1940, for the importance of this bacteriological work).

Yet Griffith is not remembered today for his contributions to the biological understanding and classification of infectious diseases but for a single "oddball" paper – his 1928 study in which he had shown that nonvirulent bacteria could be transformed into virulent types when mixed with previously heat-killed bacterial strains of a different virulent type, a phenomenon known as *transformation*. As Sir Peter Medawar (1915–1987) once put it, the behavior of pneumococci Griffith recorded evoked the notion that "they could undergo something akin to a transmutation of species" (1968). This was extremely puzzling at a time where it was still unclear whether bacteria had genes and whether they could evolve at all (see Creager, 2007).

Unsurprisingly, American researchers at the Rockefeller Institute in New York, in particular Oswald T. Avery (1877–1955), initially perceived Griffith's experimental finding as a "bombshell" in the field of immunology because it called into question the doctrine of "immunological specificity" (Dubos, 1956, p. 40; see Amsterdamska, 1993). Thanks to his reputation as an excellent laboratory scientist, however, Griffith's results were fast-replicated in several laboratories across Europe and in the United States. The hunt for the nature of the "transforming principle" culminated with the discovery that DNA was the active molecule involved in transformation (Avery et al., 1944), and placed researchers on the "path to the double helix" (Olby, 1974/1994; Morange, 1998).

Despite our familiarity with different aspects of this history, and the later developments of molecular biology that ensued (Judson, 1996; Kay, 2000), the conceptual foundations of the transforming experiment remain poorly understood and have received little historical attention

(see Eichmann and Krause, 2013 for a renewed interest in the topic). As bacterial geneticist and Nobel Prize laureate Joshua Lederberg (1925–2008) reported after trying for several years to reconstruct the "conceptual antecedents" of the transformation study: "[w]e are at loss trying to trace the intellectual influence behind his [Griffith's] experiment" (1992, p. 264).

To understand the significance of the transforming experiment, I argue, we must place it within Griffith's own research interests, which were epidemiologically-oriented as well as based on the production of detailed bacteriological knowledge. "Griffith's primary concern" with the results in the 1928 article, as Maclyn McCarty (1911–2005) emphasized, "was with their implications for the *epidemiology* and disease patterns of pneumonia" (1985, p. 77; emphasis added). Yet, "Griffith's speculations on the epidemiological significance of the interconvertibility of pneumococcal types," René Dubos (1901–1982) previously noted, have "remained virtually unnoticed, and have been largely forgotten," while "his experimental findings had an immediate, enormous impact on immunologists all over the world" (Dubos, 1976, p. 136). This paper seeks to recuperate the "epidemiological significance" of the interchangeability of pneumococcal types as studied by Griffith. Historicizing the transforming experiment will show that it cannot be divorced from the latter's wider interests in the epidemiological aspects of infectious diseases.

The significance of microbial transformation, in brief, rest not (only) on it initiating a cascade of events leading to molecular genetics but also – and perhaps especially – on its implications for epidemiological approaches based on knowledge of the biology of host–parasite interactions. Griffith's thirty years of research on changes in disease virulence, in fact, should be considered as a whole in which bacterial transformation is an important part – and following the historian of science and medicine J. Andrew Mendelsohn, certainly not some "quirky medical idea" (2002, p. 29). The experiment on bacterial transformation, it turns out, belongs to a wider scientific effort to understand the nature of variation and the origins of epidemics in the interwar period (see Amsterdamska, 2005). Understood in this way Griffith's work on the origin and disappearance of infectious diseases and the transforming experiment cease to be considered merely as the "prelude" to the discovery that genes are made of DNA. Placing his scientific contributions within a longer time period also leads us to rethink and question some of the most prevalent historical conceptions about Griffith's work.

After introducing some biographical elements concerning Griffith's life, I situate his works within the wider context of epidemiological, bacteriological, and medical research in the interwar period. I then explore his earlier study at the Royal Commission for Tuberculosis and at the Ministry of Health and I show how they are continuous with the discovery of transformation in 1928. Following a detailed examination of the transformation study and some aspects of its reception in Europe and in the United States, I turn to Griffith's later research on streptococcus diseases in relation to rheumatic and scarlet fever, conducted alongside bacteriologists and epidemiologists in the late 1920s and mid-1930s, which were of great public health significance at the time. The paper concludes with a brief examination of the unpublished correspondence between Griffith and American bacteriologist Rebecca Lancefield (1935–1937), who developed an alternative system of bacteriological classification based on precipitin-test reaction, in contrast to Griffith who subdivided types by slide agglutination technique.

Elements of Fred Griffith's Biography

"There is so little information about Fred Griffith!" Lederberg lamented (Letter from Lederberg to Pollock, 1970).[3] Most of the sources related to Griffith's work, it should be noted, are largely based on memory, not on first-hand statements or observations.[4] Reconstructions of Griffith's contributions, furthermore, often sounds rather heroic and the general lack of correspondence to and from Griffith poses a serious challenge to any comprehensive reconstitution of his life and work. The following section attempts at providing a more synthetic view of Griffith's biography that can help us gain a better understanding of his career path and the evolution of his scientific ideas on infectious disease biology.

[3] The Joshua Lederberg papers, deposited at the National Library of Medicine, can be freely accessed here: http://profiles.nlm.nih.gov/BB/. All letters to and from Lederberg, Winston Maxted, Martin Pollock, Graham Wilson, Rebecca Lancefield, Robert Olby, and Alvin Coburn, unless otherwise stated, will be quoted from this online archive.

[4] Information in this section comes mostly from obituaries and letters about Griffith, from the first four Griffith Memorial Lectures, and from Olby (1974/1994), Dubos (1976), and McCarty (1985). Griffith's personal papers and correspondence have almost all disappeared during the war, when his house was flattened, and he had no offspring. Some letters between him and Rebecca Lancefield at Rockefeller survived and are available in the Rebecca Lancefield papers at the Rockefeller Institute.

The son of Joseph and Emily Louisa Griffith, Fred Griffith was born at Hale in Cheshire in 1879. Trained in medicine, he graduated from Victoria University in Liverpool in 1901. After being physician and then a house surgeon, holding resident posts at the Liverpool Royal Infirmary, he was appointed as Alexander Fellow in Pathology at the Thompson Yates Laboratory in Liverpool in 1901, a private institution dedicated to research in biochemistry, tropical medicine, experimental medicine, and comparative pathology (Figure 1). Between 1903 and 1911, Griffith worked as a bacteriological investigator on the Royal Commission of Tuberculosis, together with Arthur Stanley Griffith and Arthur Eastwood (1867–1936). In 1910, he took the Diploma of Public Health (D.P.H.) from Oxford University and one year later he joined the Local Government Board as a medical officer at the Ministry of Health in London where he stayed until he was killed during an air raid in the Second World War.

Figure 1. Entrance of the Thompson Yates and Johnston Laboratory, Liverpool, UK, in 1903, where both Stanley and Fred Griffith worked. *Source*: The Thompson Yates and Johnston Laboratory Report, 1903, London: The University Press of Liverpool.

Arthur Stanley Griffith, Fred's older brother, was also a graduate from Victoria University, which awarded him the Derby exhibition prize at the Liverpool School of medicine. In 1901, two years after being the first Alexander Fellow at Thompson Yates and Johnston Laboratory, Stanley received his M.D. degree and the Diploma in Public Health of the Royal English College, with a medical thesis on "The flora of the conjunctiva in health and disease" (Griffith 1901). Arthur Griffith is particularly known for his work on the typing of the tubercle bacilli and for his demonstration of the potentially severe effects of bovine tuberculosis on man through milk consumption, and especially for children, a work he carried out as one of the principal investigators for the Royal Commission on Tuberculosis. Once the work of the Commission was completed Stanley continued his research on tuberculosis under the auspices of the Medical Research Council at the University of Cambridge. In 1926, he was awarded a PhD from Cambridge and in 1927 he received the Weber-Parkes prize and medal of the Royal College of Physicians for his overall contribution to the study of tuberculosis. His state of health fell out in the early 1940s and he died in 1941, a few days before his younger brother was killed during the London blitz.

After their participation in the Commission, Fred Griffith and Arthur Eastwood both accepted positions at the Local Government Board in London. In 1915 they were joined by William McDonald Scott (1884–1941), a noted bacteriologist who was in charge of investigating the spread of cerebrospinal fever in Britain. A graduate from Edinburgh in public health, Scott studied tropical medicine and hygiene before obtaining his M.D. degree in 1910. Scott and Griffith became close collaborators and also good friends. Together, they developed a new method to test for syphilitic infections (Griffith and Scott, 1920; see the details in Gilbert, 1928). As the Local Government Board Laboratories was taken over by the Ministry of Health during the First World War, both Griffith and Scott moved to Dudley House, located on Endell Street, in Soho, and converted in Pathological Laboratory.

It may be difficult to imagine the inner working of Scott and Griffith's laboratory but according to Hedley Wright, the author of one of Griffith's obituary in 1941, a foreign visitor might have been "short of appalled" to see the internal functioning of this "chaotic environment"; and yet the quality of the contributions coming out of it was widely recognized within and outside Britain (Wright, 1941, p. 588). Wright noted further that not only Griffith and Scott "did all their own bench work with very little up-to-date equipment" but that these two "could do more with a kerosene tin and a primus stove than most men could do

with a Palace'' (*Ibid.*). This is but one example of the heroic rhetoric that is often employed to describe Griffith's work.

Many have also reported on the generosity and modesty of Griffith and Scott, who were keen to assist other bacteriologists investigating infectious diseases and working in the country or abroad (Downie, 1972, p. 2).[5] In 1931, for instance, Griffith helped American bacteriologist Alvin Coburn in (1899–1975) identifying *Streptococcus pyogenes* serologically. Five years later, on the occasion of the 2nd International Microbiology Congress in London (July 25–August 1, 1936), Coburn visited Griffith at which time he took the famous snapshot (Figure 2) of Griffith and his dog Bobby on the Downs, near Brighton (Coburn, 1969; see also Dubos, 1976). The picture was sent to Oswald Avery at the Rockefeller Institute when Coburn realized ''how deeply Avery's work was founded on the discovery made by Fred Griffith'' (Coburn, 1969, p. 627). Avery framed the picture and kept it on its desk and after retirement, at his home, throughout the ''transforming'' and ''post-transforming years'' (Letter from Coburn to Lederberg, November 9th 1965, p. 2).

On the eve of the Second World War, as senior bacteriologist of the Ministry of Health, Griffith was sent to Cambridge to create and manage the Emergency Public Health Laboratory (EPHL), where he teamed with Bruce White (1891–1949), the other senior bacteriologist on the staff at the EPHL. Though some expected that Griffith would take the lead in the organization of the EPHL, and apparently ''Topley always tried to force him into this,'' Griffith was, it was said, ''without political ambition'' and preferred to leave these matters to him (Letter from Wilson to Pollock, 8th December 1969, p. 2). As it turned out, Griffith also had apparently ''no flair for organization'' and his dislike of constantly meeting new people made him want to return to London (Letter from Maxted to Lederberg, no date). Thanks to Leonard Colebrook's help, another long-time scientific collaborator, he was able to return to London where he established a streptococcal research unit at Queen Charlotte's Isolation Block at Hammersmith, which is where he began working with Stuart Dunsmore Elliott (1907–1986).

[5] ''Few public-health bacteriologists in the British Isles do not owe to Griffith and Scott gratitude for help received, some of it acknowledged here and there as a footnote to a paper, yet more unexpressed, but living in the thousand and one details of their daily life'' (Wright, 1941, p. 589). Graham Wilson also emphasized in the late 1960s that ''[a]nybody who went to them [Griffith and Scott] during life with a problem found them a mine of information, imparted in the most modest fashion and accompanied by helpful suggestions and wise criticism'' (Letter from Wilson to Pollock, 8th December 1969, p. 1).

Figure 2. Fred Griffith on the Downs in Brighton, UK. Picture taken by Alvin Coburn in 1936. *Source*: Joshua Lerberg Papers. Courtesy of the National Library of Medicine, National Institute of Health.

Upon his return in the capital, Griffith lived in his private house in Eccleston Square, in which he stayed with a housekeeper and his niece. Griffith's collaborator and friend William Scott also shared his house. When the Blitz began in April 1941, many friends and colleagues of Griffith believed they should all move out of central London at once but he apparently refused "to move for any German" (Letter from Maxted to Lederberg, no date). A bomb flattened Griffith's house a few days later, killing him, Scott, and the housekeeper. Only Bobby and the niece survived the raid. After Griffith's death, Stuart Elliott became head of the Streptococcal Research Laboratory (McCarty, 1985, p. 20).

Fred Griffith was often described as a "quiet and reticent" man who was "always happiest at the bench" (Letter from Wilson to Pollock, 8th December 1969), and "like his brother he was a recluse, known to few" (Wright, 1941, p. 588). Unlike his brother, however, he remained a bachelor while Stanley Griffith married Anna Nellie Griffith, a graduate

in mathematics and physics. Together they had a son, John S. Griffith (1928–1972), born the same year his uncle published his study on the transforming principle.[6] Fred Griffith "seldom attended meetings" and was a "classical example of the backroom boy" (Letter from Wilson to Pollock, 8th December 1969, p. 2). He disliked going to scientific conferences and was almost forced into a cab by William Scott and V.D. Allison to get to the International Congress in Microbiology in London 1936 (Olby, 1994, p. 171). During the delivery of his paper on "The agglutination of hemolytic streptococci" (1937), he was nearly inaudible as he apparently spoke too softly throughout the whole presentation (Dubos, 1976, p. 132).

Outside of his work as a civil servant in bacteriology, Griffith enjoyed walking his Irish Terrier Bobby and driving at high speed in narrow streets, a behavior that impressed and scared his American guest Alvin Coburn in 1936 (Dubos, 1976). He owned a house in Brighton that was apparently "more modern in architecture than anything in Beverly Hills" (Letter from Coburn to Lederberg, November 19th 1965), a fact standing in stark contrast with his alleged conservative attitude. Indeed, Griffith was a member of the Honorable Society of Gray's Inn and many people who have known him personally such as Winston Maxted (1911–1999) have described him as being "patriotic" – for instance, Griffith was always the first with his "aluminium pots and pans for Spitfires, and with War Bonds and in following other governmental exhortations." In Maxted's opinion, "Griffith's politics, social and professional attitudes and every aspect of his life" were conservatives (Letter from Maxted to Lederberg, no date, p. 2). In a letter to Lederberg, Coburn similarly refers to Griffith as "one of England's most conservative scientists" (Letter from Coburn to Lederberg, November 19th 1965). In a sense, this conservative attitude could be seen in Griffith's repeated emphasis on the biology of microorganisms to explain the rise and fall of epidemics. Understanding epidemics through the biology of the microorganism, indeed, is a classic image of scientific conservatism and, in this respect his work remains close (in spirit at least) to Robert Koch's postulates (see Gradmann, 2014).

The snapshot with Bobby is often considered to be the only existing photography of Fred Griffith (together with the one in Downie's fourth Griffith Memorial Lecture, 1972). Here (Figure 3), we can see him

[6] John Griffith became a theoretical chemist and biophysicist, and while working at Cambridge and Oxford, he played a critical role in calculating the helical structures of DNA for James Watson (1928–) and Francis Crick (1916–2004). Afterwards, his interest in biology and immunology led him to elucidate aspects of the nature of prions. Griffith suggested that the active agent might be an infectious protein but his death in 1972 prevented him from witnessing the confirmation of his views (Lagnado, 2005).

Figure 3. Fred Griffith enjoying a ski trip in the Alps (no date). *Source*: Picture found in the office of Rebecca Lancefield. Courtesy of Dr. Richard M. Krause, who himself received it from Maclyn McCarty.

enjoying himself during a ski trip in the Alps. Maclyn McCarty, co-authored with Avery of the 1944 paper, discovered this picture of Griffith on skis in Rebecca Lancefield's office at the Rockefeller Institute after she passed away in 1981.[7] How she came into possession of this

[7] When Griffith and Lancefield began to correspond in 1935 he was just returning from a ski trip in the Swiss Alps (Letter from Fred Griffith to Rebecca Lancefield, March 18th, Rebecca Lancefield papers, Rockefeller University, Series 450, L 221, box 3, folder 1). Griffith was a close friend to Colebrook, one of his long-term collaborators, and the two of them used to go skiing together in the Alps (Letter from Winston to Pollock, December 8th 1969, p. 2). Colebrook might have taken the picture. On Lancefield, see the biographical memoir by McCarty (1987) and O'Hern (1975).

picture is not known. But as it happened, Griffith and Lancefield had corresponded between 1935 and 1937, sending each other bottles of sera and various microbial strains across the Atlantic while reflecting more broadly on the significance of their different approaches to bacterial classificatory systems. In the last letter of Lancefield to Griffith at the Rockefeller Institute she invited him to visit her laboratory: "Why don't you make a trip over here sometime?"[8] Griffith's response is lost. After the war, it was Stuart Elliott who crossed the Atlantic Ocean and reconciled the typing methods of Group A streptococcus (GAS) used by Griffith and Lancefield (McCarty, 1985). Through his many visits to New York, Elliott became a close friend of Lancefield and supported her work throughout his life.[9]

Epidemiology and Bacteriology in the Pre- and Interwar Period

> What happens when an epidemic slowly emerges, gathers increasing momentum until it reaches its climax, and then dies gradually away until the disease falls back to the endemic level it started from? What are the respective shares of microbic virulence and host resistance? Is the decline of an epidemic due to a falling off of virulence, or to the exhaustion of susceptible materials? (Cushing, 1922, p. 429)

These foundational questions in epidemiology, formulated here in the editorial of *The Canadian Medical Association Journal* in 1922, had remained in large part of theoretical interest until the emergence of the influenza pandemic in 1918–1919, from which flowed a general feeling of powerlessness in face of its devastating effects (Mendelsohn, 1998; on the history and epistemology of influenza pandemics, see Méthot and Alizon, 2014). At the turn of the past century, answers to these questions were sought either in changes in the germ, the host, or in some other environmental factors. Typically, while bacteriologists postulated changes in the germ's virulence, epidemiologists hypothesized wider, historical changes in the social or cultural environment. The popular

[8] Letter from Rebecca Lancefield to Fred Griffith, July 30th, 1937, Rebecca Lancefield Papers, Rockefeller University Series 450, L 221, box 3, folder 1.

[9] For example: "Dear Stuart, This is to register the enthusiastic approval of everyone consulted about arranging plans to get you to join our laboratory as soon as possible for you to get away this spring and to stay until the money gives out or you feel you have to go" (Letter from Rebecca Lancefield to Stuart D. Elliott, March 7th 1979, Rebecca Lancefield Papers, Rockefeller University, Series 450, L 221, Box 9, folder 5).

hypothesis that a change in the germ's virulence or infectivity could account for the cyclical behavior of epidemics was widely discussed in the first two decades of the twentieth century in England and in the United States (Amsterdamska, 2004, p. 487).

Already in 1905, for instance, British medical statistician John Brownlee (1868–1927) argued on the basis of his study of vital statistics that "the number of cases must be due to the loss of infectivity in the germ itself, and not in the lack of individuals who may be supposed open to contagion" (1905–1906, p. 486). Brownlee, however, did not inquire into what such a biological change may be. The following year, British epidemiologist William Hamer (1858–1934), a vocal critic of bacteriology, intended to account for the waxing and waning of epidemics not as a "change in the number of the sick" but as a change in the "rate of infection and the density of the interactants" (Mendelsohn, 1998, p. 317). As Mendelsohn noted, this model rendered bacteriological, immunological, and environmental variables altogether "superfluous" in accounting for the epidemic. What mattered in Hamer's model was the continuous arrival of fresh susceptible individuals (either from birth or from migration) and the ways in which this flow of individuals could disturb a biological "equilibrium" established at the population level. Hamer's mathematical model was adopted by advocates of a more "experimental" approach to epidemiology in England, such as statistician Major Greenwood (1880–1949) and bacteriologist W.W.C. Topley (1886–1944) (Greenwood and Topley, 1926; Amsterdamska, 2004).

Despite the prominence of statistical approaches to disease in the interwar period, as historians have made us aware (Amsterdamska, 2005), it is important to underline that bacteriological studies have continued to occupy a central place in terms of methodological approaches and research questions, on the one side, and that this work was often pursued "largely outside the institutions, research schools, and journals of academic biology, in the predominantly hospital and public health spheres of a most applied and medical laboratory science," on the other (Mendelsohn, 2002, p. 31). Fred Griffith, for instance, who worked in the Pathological medical laboratory in London, did not train as a statistician but as a medical bacteriologist. He viewed changes in a germ's virulence as a fundamental factor in explaining the origin of epidemics and the geographical path of disease outbreaks more generally. For him, the constant arrival of insusceptible individuals could not provide – in itself – a complete explanation of the decline of epidemics; scientific investigators also had to consider incremental changes in the

biology of the microorganism, and how these changes, in turn, could affect the nature of its relation to the host and to the wider population.

As we will see, Griffith was convinced that modifications in the biology of the causal germ not only had consequences on illness severity at the patient level but also at the community level, as it changed the *epidemiological distribution* of diseases, that is, it brought new disease to the fore and caused others to recede in the background. Griffith's view of the origin and decline of epidemics were not based on a crude bacteriological and reductionist doctrine but on a more subtle form of biological adaptation being established between hosts and parasites. His work indicates also that bacteriology and epidemiology were perhaps much less in an antagonistic relation than what was often assumed in the historiography (on this point, see Steere-Williams, 2015). But before, let us go through some of the stages in the history of bacteriology to contextualize Griffifth's work on changes in serological types.

Conceptual Flux in Bacteriological Research: From Pasteur to Griffith

In the second half of the nineteenth century Louis Pasteur (1822–1895) had shown the phenomenon known as "virulence" or disease severity to a host could be decreased through specific attenuation techniques or again, it could be enhanced by serial host passages (Moulin, 1992). Understanding the nature and the significance of variable virulence continued to be a fundamental research problem at the Pasteur Institute as well at the Robert Koch's Institute in Berlin, at the turn of the twentieth century (Gayon, 1995; Mendelsohn, 2002). During the first three decades of the past century, in fact, the "identity" of microbial organisms was in crisis. Inherited from the last quarter of nineteenth century medical bacteriology, the doctrines of "monomorphism" and "pleomorphism" still exerted their influence on the concepts of variation and variability in microbial life forms (Mazumdar, 1995; see also Gradmann, 2000).[10]

How to reconcile "the extraordinary persistence of types" during epidemics with the "rapid transformation [of germs] under conditions favorable to change" (Hamer, 1906, p. 570), for instance, was a thorny

[10] The thesis of pleomorphism derives from the works of naturalist Karl Nägeli (1817–1891) and stipulates that all microorganisms, in different states (e.g. virulent or avirulent), form a single species. For Nägeli, microorganisms not only change morphologically or physiologically but can also *transform* into one another. In contrast, monomorphism, a thesis often attributed to Robert Koch (but cf. Mendelsohn, 2002), claims that microbial species are well-defined in nature, and cannot transform (though they admit intra-species variation).

issue at a time when the Darwinian concepts of natural selection and evolution only began to be applied to the microbial world (see O'Malley, 2009). The concept (and scope) of "biological variation," furthermore, was itself unclear and variable as reflected by its usage in the context of microbiological research at the time (see Cole and Wright, 1916, p. 212; see also Eastwood, 1923, Hadley, 1927, and Arkwright, 1929, 1931. For a historical account, see Amsterdamska, 1991, and Creager, 2007). The conceptual flux in bacteriological research was increased by the problem of delineating microbial species: not only was knowledge about bacteria still fragmentary but also traditional biological concepts applied poorly to bacteria and viruses (though the two were not distinguished yet). First, the concept of "species" was difficult to operationalize because of the peculiar mode of reproduction of microorganisms and also due to the speed at which new generation could arise. Second, the problem of sorting out microorganisms into species was accentuated due to their great variability, functionally and morphologically as well as with respect to pathogenicity or virulence to hosts (see, Topley and Wilson, 1934).

Initially, claims about the possibility for bacteria to undergo physiological and morphological changes were regarded as suspect although they exerted a clear fascination for researchers. As Ludiwk Fleck (1896–1961) once commented, he used to regard words such as "vitalism" in biology, "specificity" in immunology, or "bacterial transformation" in bacteriology as "slogans" endowed with "magic power" (Fleck, 1979, p. 43). After bacteriologist Joseph Arkwright (1864–1944) from the Lister Institute in London correlated the agglutinative behavior of enteric bacteria to changes in virulence, the interest in studying bacterial variation grew steadily (Amsterdamska, 1991, p. 193). Fred Griffith, for one, was very curious about biological variation and wondered whether it was an artefact or a "real" feature of biology (Letter from Coburn to Lederberg, 1972, p. 2). Yet even if the existence of the diversity of microbial variability was progressively recognized, the distinctions between genetically stable variability (i.e. the result of mutations) and momentary variations (i.e. the result of adaptation to changes in the environment) were not firmly established as far as 1927 (Löwy, 1988, p. 234). In fact, until the mid 1940s, the possibility of drawing up an "operational distinction" between mutation and adaptation did not seem possible to most microbiologists (Hotchkiss, 1966, p. 182/2007; Creager, 2007).

Paramount examples of virulence modification with important implications from a medical point of view included colonial changes from "smooth" (S) to "rough" (R) described by Arkwright for the dysentery group (1921, 1929, 1930, 1931) and also by Griffith (1923). Indeed, these

gradual changes from S to R correlate with loss of virulence and altered sensitivity to bacteriophage. The loss in virulence was due to the disappearance of a polysaccharide capsule (giving the culture a ''rough'' outlook as opposed to a ''smooth'' or shiny, almost watery one) – which permits bacteria to avoid phagocytosis – and corresponding antigenic components. Directionality of changes in colonial aspects as described by Arkwright was thought to go from smooth (virulent) to rough (avirulent), a thesis Griffith's 1928 paper on transformation called into question (Topley and Wilson, 1934). Yet what was the nature of those observable changes in bacterial colonies? Were they heritable, analogous to de Vries' notion of ''mutation,'' or were they transient ''physiological'' adaptations? Clarifying the nature of those changes, Griffith (1928, 1934) and others hoped, would help explaining the incidence of diseases such as pneumonia at the epidemiological level (Olby, 1994, p. 170).

Pneumonia and the "Fixity" of Serological Types

Issues concerning the nature of changes in microorganisms were intimately connected to the practical possibilities of developing effective treatments against infectious diseases, particularly serum therapy. Since Emil von Behring (1854–1917) and Shibasaburo Kitasato (1853–1931) demonstrated the possibility to cure sick animals using anti-serum previously obtained from animals immunized with the corresponding bacterial toxins, much effort was devoted to producing antipneumococcus sera and other therapeutic agents that would be useful against a number of infectious diseases (Gradmann and Simon, 2010) including pneumonia, a significant cause of death and morbidity (Podolsky, 2006).

While earlier attempts at producing effective serum-therapy were unsuccessful, German bacteriologist Fred Neufeld at the Robert Koch Institute in Berlin made a major theoretical advance of practical importance. Neufeld believed that the classification of pneumococcus into serological types was at the foundation of the science of immunity. Together with his coworkers, he established the existence of immunologically distinct serological types of pneumococci in 1909, showing that those pathogens do not form a homogenous group (Neufeld and Händel, 1909). In 1912, they delineated three such distinct groups of pneumococci (on Neufeld see, Mendelsohn, 1998, and Eichmann and Krause, 2013; on the standardization of serum therapy see Mazumdar, 2010, and Hüntelmann, 2010).

Simon Flexner (1863–1946), then director of the Rockefeller Institute in the United States, took notice of Neufeld's work. Inspired by his results, Flexner and Rufus Cole (1872–1966) provided much of the impetus to

classify the disease-causing organisms involved in pneumonia outbreaks, and to look for the production of effective and standardized serum. To organize pneumococcus research at the Rockefeller Institute, Cole appointed a talented chemist, Oswald Avery, in 1913. For Avery, like for Neufeld, the chemical basis of immunological specificity and the differences in antigenic properties provided not only a "reliable method" to determine the variety of bacterial strains but also "the only rational basis for the study of immunotherapy in pneumococcal infection" (Avery, 1915, p. 816). Avery stressed that serological types are based on "well defined immunological differences" that reflect "the extraordinary uniformity and comparative fixity of the specific groups" (Avery, 1915, p. 816). Upon his arrival at the Rockefeller, Avery joined Alphonse R. Dochez (1882–1964) with whom he reported on the relation between pneumococcal types and human pneumonia (Dochez and Avery, 1915). In *Acute Lombar Pneumonia- Prevention and Serum Treatment* (1917), Avery, Chickering, Cole, and Dochez confirmed Neufeld's results. The American team identified four Types after the examination of cases of pneumonia: Types I, II, III, and a heterogeneous named Type IV. In London, Griffith (1922) obtained the same distribution of pneumococcus as the one observed by the American researchers in New York. In addition, Griffith identified twelve serological types within the Type IV group (Downie, 1972, p. 2).

Over the years, Avery and Griffith have looked at each other's work with great respect although they never met and (probably) never corresponded either.[11] Commentators have noted similarities between the two bacteriologists: Both were interested in streptococci and pneumococci; they were both bachelors; in terms of scientific practice, both were modest and meticulous with their experimental work[12] as well as generous with their time in helping others; not to mention that they were almost "obsessively cautious" in drawing conclusions (Pollock, 1970, p. 10). Avery and Griffith, however, entertained different views about the biological and clinical meaning of type variation in pneumococcus: "For Avery the Types were distinct and separate forms, almost with the status of species, while for Griffith they were unstable varieties" (Russel, 1988, p. 395).

[11] "I probably was the source of Dr. Elliot's 'definite opinion' that Dr. Avery and Dr. Griffith never met or corresponded. I can only say that this certainly has always been my impression and was doubtless casually stated around the laboratory" (Letter from Lancefield to Lederberg, December 12th 1972).

[12] Bacteriologist Stuart D. Elliott, who worked with Griffith for several years, noted that he was "fanatic about techniques" so much so that his meticulousness "sometimes aroused the exasperation, if not the fury, of his associates and assistants" (cited in Dubos, 1976, p. 132).

Avery's belief in the doctrine of immunological specificity explains, at least partly, why he was at first reluctant of accepting Griffith's claim that pneumococci can change in their immunological signature (see Dubos, 1956). And while Avery refrained from speculating about the evolutionary origins of serotypes, Griffith did not hesitate to explore "in the light of evolutionary possibilities" the transformation of one type into another (1917, p. 185).[13] Examining Griffith's research prior to the transforming experiment and well as the 1928 study, the following sections will illustrate his constant preoccupation with finding changes in microorganisms that could explain the origins of epidemics and the ecological paths of disease. Beginning with his work as part of the Royal Commission on Tuberculosis and continuing throughout his career while at the Ministry of Health in London, Griffith's interest for changes in microorganisms also provides insights into his dynamical views of bacterial species and the evolution of serological races in nature. Taking a longer view of his scientific career will help us see that Griffith was not "convinced of the fixity of bacterial types" until as late as 1928, contrary to what was often supposed (Letter from Wilson to Pollock, December 8th 1969; see also Pollock, 1970, Downie, 1972) but that he contemplated the possibility of bacterial transformation much earlier on.

Griffith's Bacteriological Research Before the Transforming Experiment

The Royal Commission on Tuberculosis (1901–1911)

First identified by German bacteriologist Robert Koch (1843–1910) in 1882, American bacteriologist Theobald Smith (1859–1934) claimed, fourteen years later, the existence of distinct bovine, avian, and human

[13] For example, in a paper on the classification of pneumococci, Avery wrote: "In the present discussion no attempt is made to interpret the experimental data in terms of their phylogenetic significance. Whether the subvarieties of the second group of pneumococci represent strains which have acquired independently certain adaptive characters, or whether they are related to each other and to the fixed type by the *lineage of common descent* is interesting. However, the limited nature of the present study *precludes the formulation of any hypothesis as to origin*" (Avery, 1915, p. 818; emphasis added). In contrast, as part of a study of pneumococcus, Griffith concluded: "[In] *the light of evolutionary possibilities* […] one might *speculate* on the relationship to the meningococcus group of the naso-phrayngeal organism which cannot be accepted as a meningococcus but differs culturally from the true meningococcus only in the marked production of pigment. […] According to one's point of view, such strains might be considered as the possible starting point or the possible end point *in the process of evolution* of the meningococcus" (Griffith, 1917, p. 185; emphasis added).

types of tuberculosis bacillus – challenging Koch's views of a unique tubercle germ (on Th. Smith, see Méthot, 2012). During the British Congress on Tuberculosis in London in 1901, Koch responded that the bovine form of tuberculosis was not dangerous for human populations, precisely *because it differed* from the human type (Rosenkrantz, 1985). In the debate that ensued among participants the government conveyed the Royal Commission on Tuberculosis, which was appointed on August 3rd 1901 under the chairmanship of Sir Michael Foster (1836–1907). Its purpose was to determine (1) "whether the disease in animals and man is one and the same; (2) whether man and animals can be reciprocally infected with it; and (3) under what conditions, if at all, the transmission of the disease from animals to man takes place, and what are the circumstances favorable or unfavorable to such transmission" (Ritchie, 1907, p. 3; see Francis, 1959).

The commission conducted two parallel investigations of the tubercle bacillus in human and animal forms at two experimental farms in Stansted, Essex, lent by Sir James Blyth (1841–1925), a British businessman. A third series of comparative experiments was then carried out in laboratory. Stanley Griffith, Arthur Eastwood, and Louis Cobbett (1863–1947) were the senior resident investigators on the Royal Commission. They were joined by Fred Griffith. During the investigation, the two brothers worked closely together. Studying the cultural characters of the bovine tubercle bacillus, they conducted a large number of modification experiments of virulence with bacilli of bovine and human origin and reported on their effects in different species including calves, goats, monkeys, mice, rabbits, pigs, and guinea-pigs after subcutaneous inoculations (Griffith and Griffith, 1907). During ten years, the commission published a series of detailed interim reports (that appeared in 1904, 1907, 1909). As recommended by the Royal Commissioners, comparative pathologist Theobald Smith's experiments used to distinguish the bovine from the human form were repeated. While he was able to confirm Smith's general statement about the different behavior of virulent tubercle cultures of human and bovine origins, Stanley Griffith concluded that this did not point to a physiological difference between the two (1907, p. 57).

"Man," the final report concluded, "must therefore be added to the list of animals notably susceptible to bovine tubercle bacilli" (Final Report of the Royal Commission, 1911, p. 122).[14] As to the question of the identity

[14] I am quoting from the summary of the report published in the *British Medical Journal*. The complete report appeared in Her Majesty's Stationary Office and was led by Sir William Henry Power (1842–1916), who acted as Chairman of the Commission from from 1907 after the death of Sir Michael Foster. The whole report contains various appendix written by Stanley Griffith, Fred Griffith, Arthur Eastwood and Louis Cobbet.

of the disease, the commissioners considered that the human and bovine types are "varieties of the same bacillus" and consist in "one and the same disease" (*Ibid.*, p. 123). Concerning the possibility of reciprocal infection they stated that "mammals and man can be reciprocally infected with the disease" (*Ibid.*); and as to the conditions facilitating the transmission, they concluded that they are mainly dependent on the "susceptibility" of the animals and the opportunities of transferring the infection to man (*Ibid.*, p. 124). From a public health perspective, the authors of the report called attention to the danger of drinking infected cow milk.

Of special interest here is the question of the difference of virulence between bovine and human tubercle bacilli towards certain animals and not others. Why, the commissioners asked, is the human bacillus more virulent in monkeys than in goats, calves, and pigs? Is the human form a "modified" or "degraded" form of the bovine tubercle? Is its degradation permanent? Drawing upon the works of the Griffith brothers, the commissioners noted the following: "[R]epeated attempts were made to *transmute bovine into the human bacillus*, and vice-versa" (Final Report of the Royal Commission, 1911, p. 124; emphasis added). Most of these experimental attempts, several of which were performed by Fred Griffith (1911), were unsuccessful, however, and lent support to the claim that both types were "remarkably stable." The authors conceded that "transmutability of bacillary type" is "exceedingly difficult, if not impracticable of accomplishment by laboratory procedures." In spite of the failure to experimentally transmute the bacillary type, the commissioners also stated clearly that they were "*not prepared to deny that the transmutation of one type into another may occur in Nature*" (Final Report of the Royal Commission, 1911, p. 123; emphasis added).

This conclusion calls into question the view put forward by Downie (and others) in the Fourth Griffith Memorial Lecture, that prior to 1928 "Griffith was conditioned to believe that bacteria existed in immutable types" (1972, p. 3).[15] Indeed, although the tuberculosis bacillus appeared remarkably stable and resistant to experimental transformation, the investigators did not give up the possibility of transmutation *in general*. The several experiments designed to test whether the human type of tubercle bacillus could be transformed into a bovine type (and vice-

[15] According to Graham Wilson, "Many years before [the transforming experiment], he [Griffith] had worked with his brother Stanley on the tubercle bacilli, and was convinced of *the fixity of the mammalian types*; and yet here he had produced a change in the types of pneumococci" (Letter from Wilson to Pollock, December 8th 1969). One year later, Pollock nuanced Wilson's words, however: "At the time, Graham Wilson has pointed out, he [Griffith] was firmly convinced of the fixity of bacterial types – at least in so far as the mammalian tubercle bacilli were concerned" (Pollock, 1970, p. 7).

versa) in fact speak against the claim that Griffith was "conditioned" to believe in the immutability of bacterial types. On the contrary, it shows that this possibility was taken very seriously by both the commissioners and the scientific investigators. Interestingly, such case of experimental transmutation is what Fred Griffith thought he observed at the Ministry of Health in London a little over fifteen years later.

Studies on Meningococcus and Pneumococcus at the Ministry of Health: 1917–1923

Fred Griffith's conception that serological types might evolve over time emerged more clearly in his 1917 lengthy report on meningococcus in the nasopharynx prepared for the Ministry of Health, while he was working at the Pathological Laboratory in London. In this report, Griffith confirmed the existence of two main groups based on serological type reactions. The differentiation in their antigenic capacity, Griffith noted, correlates with differences in virulence: complex antigenic structures are found in more virulent strains, whereas simpler ones (from an antigenic point of view) are localized in milder strains, usually found during the declining phase of a disease outbreak (1917, p. 176).

Griffith observed further that these groups are not "fixed types" but may be divided into sub-groups, which he called "centers of variation" in the "different stages of evolution of the meningococcus antigens" (1917, p. 175). Such variations in antigenic properties, Griffith added, must depend in the end on their "chemical constitution." Drawing on the work of microbiologist Frederick William Andrewes (1859–1932), he suggested that the latter's attempt to define species on chemical grounds "could be applied to the evolution of racial types within a species just as much as to the evolution of different species" (1917, p. 175).[16] The tendency for a bacterial species to differentiate itself into races," Griffith suggested, could more broadly "be regarded as a continuation of the same evolutionary process whereby bacteria with the characteristic of a common genus exhibit emergence into a greater or smaller number of species " (1917, p. 175). According to Griffith, these (evolutionary) considerations have implications for the origin of meningococcal types as found during an epidemic. In relation to the 1915 epidemic he was investigating, he noted that it could be due to the introduction into the country of a virulent and complex strain (from an

[16] For Andrewes, "the minute structural differences in the configuration of the protein molecule have arisen by natural selection – as a result of the chemically fittest molecules in the struggle for existence of the organisms to which they belong" (1914, p. 13).

antigenic point of view) which, owing to its passage from individuals to individuals, ''resolved'' progressively into the two less complex Group I and Group II he identified (1917, 183). Alternatively, the emergence of meningococcal epidemic might be the result of the spread of ''saprophytic [...] individual races'' which have increased in virulence alongside the domestic and harmless strains. The two main types could then have evolved simultaneously although ''one has to imagine some influence in action which sets in motion the process at a number of foci'' (1917, 184). Griffith concluded from all this that the decision between the two hypotheses ''rests probably with epidemiology'' but he hoped that the evidence he presented regarding the variations and change in the serological characters among meningococcal types ''may perhaps be of some assistance'' (1917, 184).

Pursuing other bacteriological investigations at the Ministry of Health, Griffith published a report in 1922 in which he emphasized the need to produce a uniform technique of diagnosis of types of pneumococcus. Such classification mattered for therapeutic and epidemiological purposes. As to the former, the study of the variety of types is important because antiserum is type specific (i.e. it must be produced for each type). As to the latter, a close examination of the serological types occurring in different bacterial species ''may some day provide an explanation why a ubiquitous and apparently harmless organism may suddenly become more pathogenic for its host and of such high infectivity as to propagate an epidemic'' (1922, p. 20), a point to which Griffith would return in his 1928 study.

In the course of this report, Griffith noted that during an infection, patients infected with pneumococci had a tendency to be infected by the more invasive Type I and Type II and later by Type IV (renamed ''Group IV'' by Griffith). Thus, the question arose as to whether the different strains found in a single patient were due to multiple infections or to mutations. While the American hypothesis was that ''the Type I strains die out,'' Griffith's alternative view was ''that the virulence of Types I and II becomes attenuated during convalescence, and that this change is accompanied by *mutation* of type characters, which now become degraded into those of the heterogeneous and less virulent group termed IV'' (1922, p. 35; emphasis added). Despite a number of experimental difficulties in testing for his hypothesis, Griffith maintained that it is ''theoretically possible that a mutation may occur in nature, though it cannot be reproduced *in vitro*'' (*Ibid.*, p. 36).

In 1923, when Griffith reported on his discovery of the S (smooth) and R (rough) forms of pneumococci that could account for such sudden changes, he cautioned that they were not limited to laboratory

conditions but could ''arise under natural conditions, as, for example, during convalescence from pneumonia'' (1923, p. 11). More precisely, he regarded the alteration from S to R form as a ''natural tendency'' of many species of bacteria attributable to ''degenerative changes'' and the loss of ''antigenic qualities'' – a change he would later (1928) come to describe in terms of ''vital adaptation,'' however. Referring to his 1917 report on meningococcus he linked the alteration from S to R form to a reduction in antigenic complexity (1923, p. 11).

The observable variation in bacterial cultures made a strong impression on Griffith who considered it to be the norm rather than the exception: ''the conception of a 'pure culture' of a bacterium as a number of absolutely identical individuals is no longer tenable'' (1923, p. 1). Variation in races, still, was apparently bound within the limits of the species. ''The various races of pneumococci resemble each other so closely in appearance of colonies and in the characteristic of bile solubility,'' Griffith had argued, ''that there can be no doubt that they belong to one species'' (Griffith, 1922, p. 32). This opinion was shared by his mentor Arthur Eastwood for whom ''bacteria 'breed true' to their species''; ''any variations which occur are within the limits of the species.'' According to Eastwood, ''the possibility of transmutation of species'' was not supported by evidence (Eastwood, 1923, p. 17). At that point, thus, Griffith had not directly challenged the concept of type specificity: changes from S to R remained within the boundaries of the same type. Yet he certainly had explored the possibility of transmutation when studying the changes in type characters in his 1922 report (see Olby, 1994, p. 172).

To sum up, Griffith's work conducted while he was a member of the Royal Commission for tuberculosis as well as his research on meningococcus following his appointment at the Local Government Board shows that he conceived of serological types not as fixed but as biological entities subject to various changes over time. The important point is that, Griffith was open to the possibility that bacterial types might undergo transformation long *before* he published his study of the ''transforming experiment'' in 1928. Indeed, he contemplated the possibility in the evolution of microbial species as early as 1911 although such an empirical case was not encountered (or ''produced'') prior to the study published in 1928. His interest in the evolution of serological types in nature, together with the possibility of encountering mutations and changes of types, was nurtured throughout his career at the Pathological Laboratory in London. Griffith, thus, was better prepared to accept and interpret those results in light of his earlier studies than what most commentators have claimed.

Bacterial Transformation and the Origins of Epidemics

Let us now turn to the 1928 study. When publishing his lengthy report in the *Journal of Hygiene*, Griffith had already moved on to the investigation of streptococcal infections outbreaks (to which we shall return later), but in the course of other inquiries he was made aware of new data. One fact that greatly interested him was the progressive diminution of cases of pneumonia attributed to Type II pneumococcus and the corresponding increase of cases in the heterogeneous Group IV. Between 1920 and 1927, Griffith noted, Type II declined from 26 to 4 cases, while in Group IV cases rose up from 30 to 53 (1928, p. 114). Griffith also observed that distinct serological varieties are often found in the sputum of a single patient, particularly Type I and Group IV. Why was there so much variation in strains found in single individual patients? This question had practical implications, notably for the therapeutic development of type-specific sera, which was actively pursued at the time and demanded a precise knowledge of the identity of the causative germ.

The English bacteriologist considered three alternative explanations: (1) The patient was a carrier of Group IV who became infected with Type I and developed pneumonia; (2) the patient carried a strain of the normal Group IV but because of conditions "favorable to mutation" a Type I pneumococcus "evolved in his air-passages," initiating pneumonia. On this hypothesis, Griffith noted, "the different serological types would be evidence of the progressive evolution"; (3) and finally, the Group IV strains, of lower infectivity and of less complex antigenic structure, might have derived from the Type I in the course of resistance against Type I strain (1928, pp. 114–115). As McCarty observed, it is difficult to understand why the first hypothesis was so unacceptable to Griffith, knowing that "the dissemination of several serological types throughout the population was common at times of high incidence of pneumonia" (McCarty, 1985, p. 73).[17] Nevertheless, this skepticism proved useful as Griffith focused especially on the latter two hypotheses; and it is while testing for them that he discovered (or generated) transformation.

The transformation study describes in detail several experiments conducted with mice by Griffith. In the first pages of the article, the author draws attention to the peculiar behavior of some pneumococci with a rough aspect, typically a "distinguishing feature" of an avirulent colony. Griffith, indeed, had identified a rough train associated with

[17] "On a balance of probabilities, interchangeability of type seems a no more unlikely hypothesis than multiple infection with four of five different and unalterable serological varieties of pneumococci" (Griffith, 1928, p. 117).

"the absence of virulence" that produced "typical rough colonies" but was "nevertheless able to multiply in the mouse and cause fatal septicaemia" (1928, p. 117). Observing the blood of the mouse in which a strain derived from the sputum of a case of lobar pneumonia was inoculated, it showed "pneumococci with well marked capsules, and on plate cultures rough colonies grew, identical in appearance, except in one instance, with those of the original strain" (1928, p. 117). Griffith investigated the "unusual occurrence" as follows:

> The original colony in broth was plated and on the plate three different varieties of colonies were identified. Two varieties were smooth, one of which was found to agglutinate with Type I serum and the other with Type IV (Pn. 160) serum; the third variety was rough. Four of the rough colonies were subcultivated and each was inoculated subcutaneously into a mouse in a dose of 0,25 c.c. of broth culture.

What happened following the inoculation was certainly surprising:

> Three of the mice were well when killed three days later and cultures were grown from the seat of inoculation; each culture thus obtained was inoculated intraperitonally into a second mouse without causing any ill effects. The mouse inoculated with the fourth colony *died of pneumococcal septicemia* (1928, p. 118; emphasis added).

The finding concerning the mouse inoculated with the fourth colony was surprising because it seemed to suggest a reversion of the most unusual kind, and which proved fatal for the mouse: the change in character went from "rough" to "smooth" whereas such changes had always occurred the other way around. Bacteria, it was thought, could loose their polysaccharide capsule but were unable to reconstruct one anew. Performing a series of passage experiments in mice, Griffith confirmed his initial result and noted that the change from a rough-like colony to a "much smaller shiny colony of almost water consistency was *very striking*" (1928, p. 119; emphasis added). The phenomenon of reversion from rough to smooth and the experimental procedures used to produce it are then discussed in much detail on several pages (see pp. 125–129).

The mechanism underpinning this change, according to Griffith, was the retention by some strains of "a remnant of the original S antigen" that, in small quantity was unable to cause disease but could exert a pathogenic effect when a considerable dose is injected under the skin of the animal (1928, p. 129). This mass "forming a nidus," he assumed, could also protect the strain from the defense mechanisms of the mouse body (1928, p. 130). In Griffith's words, the S antigen "may furnish a

pabulum which the viable R pneumococci can utilize to build up their rudimentary S structure'' (1928, p. 130; see also p. 153).[18] Griffith equally took notice that some strains could revert more readily than others and inquired into the conditions facilitating or hindering reversion, noticing, for instance, that the development of the S form was favored by the injection of a large dose of the R form under the skin.[19]

We do not know why exactly Griffith decided to mix dead, virulent pneumococci with living, avirulent ones into mice bodies; but it is during this part of the investigation that ''transformation'' was historically produced under laboratory conditions.[20] Griffith performed several more experiments with mice in which he inoculated ''attenuated R pneumococci together with virulent S culture killed by heat'' (1928, p. 129). ''The rough attenuated Type II culture,'' Griffith wrote, ''has apparently in two instances been changed into a virulent Type I'' (1928, p. 134).[21] Careful with such strange results, Griffith cautioned they might be due to some Type I pneumococci that survived heating. Launching into more control experiments and sterility tests to verify this possibility, he established that no organism could have survived the procedure (1928, p. 154).[22] Thus, not only was there a reversion from R to S but ''there seems to be no alternative to the hypothesis of transformation of type'' (*Ibid.*; see also Dubos, 1976, and McCarty, 1985, for details).

Griffith derived epidemiological lessons from his bacteriological study. In the discussion part of the report, Griffith noted how the serological analysis of bacterial species had ''obvious practical appli-

[18] ''When the R form of either type is furnished under suitable experimental conditions with a mass of the S form of the other type, it appears to utilize that antigen as a pabulum from which to build up a similar antigen and thus to develop into an S strain of that type'' (Griffith, 1928, p. 153). The current explanation is that bacteria, through a form of lateral gene transfer, can pick up DNA from the environment, integrate it within their own genome, and pass down this new genetic information to the next generation (Sapp 1994, p. 158).

[19] ''By S substance I mean that specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate'' (Griffith, 1928, p. 151).

[20] There might be prior experiments of transformation to Griffith's in the 1920s. See Travassos (1979).

[21] ''The injection of heated virulent S culture of Type I into the subcutaneous tissues of mice together with an attenuated R strain derived from Type II apparently results in the conversion of the latter into a virulent S culture of Type I or of Type II'' (Griffith, 1928, p. 140).

[22] ''The method by which transformation of type has be secured consists in heating to 60° for 15 min up to 3 h a virulent culture of one type and inoculating a large amount of the heated culture under the skin of a mouse together with a small dose of the R strain derived from another type'' (Griffith, 1928, p. 154).

cation in bacteriological diagnosis,'' for instance in the development of therapeutic sera for lobar pneumonia (1928, p. 148). He also considered, however, that the findings he was reporting could bear on ''others issues, probably of greater importance'' such as ''the occurrence and remission of epidemics,'' ''the appearance of epidemic types in certain diseases,'' and the ''attenuation of the infecting agents'' (1928, p. 148). What is the ''meaning'' of the types defined by serologists, he asked. Do they represent ''stages in the normal life history of a bacterium'' or are they the ''response on the part of the bacterium'' to changes in the host? (1928, p. 148) A solution to these questions, Griffith considered, ''would be a valuable contribution to the epidemiology of disease'' as it would ''explain some of the phenomena in the rise and fall of epidemics'' (*Ibid*.) Claiming that ''a Type I could be changed into a Type II or III,'' Griffith said, ''would have been received with greater skepticism than at the present day.'' Since it was shown that a pneumococcus can be attenuated, ''deprived of its type characters and virulence,'' which can then be restored under favorable conditions, however, ''the possibility appears less unlikely'' (1928, p. 154). It is thus the discovery of reversion from R to S that supported the possibility of bacterial transformation itself.

How does this relate to the hypotheses set out at the beginning? Here, Griffith remains a little unclear. ''The apparent transformation,'' he says, ''is not an *abrupt change* of one type into another but a process of evolution through an intermediate stage, the R form, from which the type characters have been obliterated'' (1928, p. 154; emphasis added). This phrasing seems to support the second hypothesis (i.e. mutation), and the author then adds immediately that ''[m]utation of type among disease-producing bacteria is a subject of obvious importance in the study of epidemiological problems'' (1928, p. 154). However, Griffith settled for the third hypothesis, namely that Group IV types are derived from Type I. Although it had remained ''purely speculative'' so long as the instability of pneumococcal types was not demonstrated, this hypothesis could now be stated with more confidence: ''the chief types revert to the Group IV varieties from which they were derived during the development of the disease in the individual'' (1928, p. 155).

Griffith conceived those changes as transient, physiological adaptations. ''The formation of a Group IV strain from Type I,'' he explains, ''might be considered as an *adaptation* on the part of the Type I pneumococcus to the altered conditions consequent on the development of immune bodies'' (1928, p. 156; emphasis added). Whereas most writers had regarded the reversion to the R form as a ''degenerative

change'' (1928, p. 156), and while it implies ''some sacrifice of its [Group IV] antigenic complexity'' Griffith interpreted this change as a ''vital adaptation,'' as suggested previously by bacteriologist Philip Hadley (1881–1963) (Hadley, 1927, p. 156; see Amsterdamska, 2004). Reversion to the R (avirulent) form, thus, is not admitting defeat on the part of the pneumococcus but an illustration of the efforts allowing the microorganism to actively develop virulent potentialities anew in the future. This biological adaptation, for Griffith, was to be understood as ''the final stage in the struggle of the bacterium to preserve its individuality [...] against adverse cirumstances'' (1928, p. 157). This is one of the most important aspects of the 1928 paper for Griffith, as it could explain why an apparently harmless saprophyte could suddenly turn into a virulent pathogen and vice-versa.

Going back to the tensions between epidemiologists and bacteriologists introduced earlier, Griffith located the cause of the decline of an epidemic in the biology of the germ itself, that is, in the identity of the causative microorganism. The possession of the S antigen, he argued, is potentially capable of transforming a harmless saprophytic, rough-like colony into a virulent smooth type endowed with what he called the ''full equipment of virulence'' of another type (1928, p. 157). Transformation via an intermediate stage S offered an appealing mechanism to account for the distribution of virulent and avirulent diseases in a population. Beyond the interest in bacterial transmutation itself, this experiment, overall, provided Griffith with an explanation of epidemic outbreaks that was epidemiologically significant and grounded in bacteriological knowledge, as the conclusion of the paper makes plain:

> The experiments on enhancement of virulence and transformation of type suggest an explanation of the manner in which pneumococcus residing as an apparently harmless saprophyte in the nasopharynx acquires disease producing powers. So long as it retains certain potentialities, indicated by the possession of traces of S antigen, the most attenuated pneumococcus may develop the full equipment of virulence. These considerations which relate to an individual case of pneumonia are capable of application to an outbreak of epidemic disease in a community. Thus the consequences which ensue on the decline of an epidemic are not only an increase in the number of insusceptible individuals but also an *alteration in the character of the infective organism* (1928, p. 157; emphasis added).

Replicating the Transforming Experiment in Europe and the United States

Historians have long been interested to know whether Griffith had any understanding of the phenomenon he produced; and most concluded that his discovery was a case of "pure serendipity." [23] It has also often been said that Griffith left to others the task of sorting out the nature of the transforming principle and that he never returned to the problem himself. [24] While it is the case that Griffith focused on other problems in biology and medicine after 1928, he did not entirely leave transformation aside. In "Serological Races of Pneumococci. Significance of Types," a paper which appeared in 1929 in *A System of Bacteriology in Relation to Medicine*, he discussed "whether pneumococci may change from one type to another under natural conditions" (1929, p. 209). Although this had not been demonstrated in human infections, Griffith said, "it is difficult to conceive that the chief types, as well as the innumerable varieties of Group IV, are absolutely fixed and inalterable" (*Ibid.*). To make his point, he referred again to the variety of Group IV strains found in the sputum of convalescent patients. Rejecting the consensus according to which the chief types "die out" he advanced the view (as in the 1928 paper) that under the influence of the immune S substance formed during the recovery phase, the pneumococcus can take on different serological characters: "Group IV strains may be derived from one or other of the chief types" (1929, p. 209).

[23] Graham Wilson claimed that he "doubted whether Fred Griffith ever realized the greatness of his transformation discovery" (Letter from Wilson to Pollock, December 8th, 1969, p. 1). "Griffith seemed to have had little idea of how this transformation came about, nor even of its great and ultimate significance," Martin Pollock wrote in the Third Griffith Memorial Lecture. "His discovery," he continued, "was a classic instance of *pure serendipity*" (Pollock, 1970, p. 8; emphasis added). Similarly, for Brock, "Fred Griffith's paper is a classical example of *serendipity*" (1990, p. 224; emphasis added). See also Chen (2010).

[24] In the First Griffith Memorial Lecture, microbiologist and geneticist William Hayes wrote: "The story of this discovery [of transformation] is told in his [Griffith's] 1928 paper, which is *the only one he wrote on this topic*. […] [N]o further references to it appear among his rather scanty subsequent publications" (Hayes 1966, p. 386; emphasis added). Brock also contends that "Fred Griffith's observations on type transformation in pneumococci are embedded in a lengthy paper on characteristics of pneumococcus types (Griffith, 1928), *the only paper* Griffith published on transformation" (1990, p. 218; emphasis added). "My guess is," American microbiologist Alvin Coburn once told Lederberg "that Fred Griffith thought little of his experiment" (Letter from Coburn to Lederberg, April 25th, 1966).

Griffith continued and discussed a number of "modifications experiments," while making clear reference to his own 1928 paper. Citing works by Morgenroth et al. (1925) in which the authors were apparently able to "transform pneumococci into streptococci" Griffith went on to describe the method he had used to show that "one type of pneumococcus can apparently be converted into another" (1929, p. 209). Detailing another case of transformation "in which a rough Type II was changing into a virulent Type I" he referenced the use of his personal method by Fred Neufeld and Levinthal (1929, p. 210). In 1927, Neufeld visited Griffith in London. During his visit, he was informed of the intriguing results of Griffith and the experimental procedures employed. Once back in Berlin, Neufeld replicated the experiment and confirmed Griffith's initial results. This explains why he and his colleague Walter Levinthal (1886–1963) published their results a few months only after Griffith's own paper appeared in print (Neufeld and Levinthal 1928).[25]

Griffith's experimental results, though not immediately endorsed, were regularly discussed at scientific meetings, in journals and in encyclopedic works, and their implications for genetics were also explored (on this see Olby, 1994). In his *Bradshaw Lecture* for example, Arkwright reported on Griffith's finding, which, "if fully corroborated will mean a great advance in the theoretical position" (1929, p. 968). During the 1st International Congress of Microbiology in Paris, he characterized Griffith's work on transformation as being of "fundamental importance in the theory of variation and the change of virulence of bacteria and the progress of epidemics" (Arkwright, 1931, p. 155). Topley and Wilson, in their *Principles of Bacteriology and Immunity* (2nd edition), wrote that to "transmute a smooth Type I pneumococcus, via the non-capsulated rough variant, into a smooth Type II or Type III pneumococcus," as suggested by Griffith, is "so important, in its biological interest and implications" (1934, p. 209). One such implication was pondered by geneticist Theodosius Dobzhansky (1900–1975) in the 2nd edition of his *Genetics and the Origins of Species*, who added: "If this transformation is described as being a genetic mutation – and it is difficult to avoid so describing it" (1941, cited by McCarty in a Letter to Lederberg October 11th, 1972; see Olby 1994; Morgan, 1944).

[25] "We used the one-cell cultures to reproduce the important experiment of F. Griffith on the transformation of avirulent pneumococci (R-forms) into virulent (S-forms) of the same but also of a foreign type […]. The communication of Griffith just appeared in the *Journal of Hygiene* (1928, Vol. 27. p. 113); owing to the kindness of Dr. Griffith, who communicated his results to one of us during a visit […] in London, we were enabled to begin with our experiments before [Griffith's paper appeared]" (Neufeld and Levinthal, cited and translated in Eichmann and Krause, 2013, p. 2233).

The central problem raised by bacterial transformation was the realization that changes in serological types entailed that the doctrine of immunological specificity had to be reconsidered (Amsterdamska, 1993). Oswald Avery's initial skepticism, thus, was likely due to the fact that he devoted much of his professional life developing this doctrine that Griffith's findings were calling into question (Dubos, 1956, p. 40; Downie, 1972, p. 3). In the United States, Reimann (1929) and Dawson (1928, 1930) successively confirmed Griffith's results. Dawson and Sia (1931), then, replicated the transforming experiment in the test-tube, succeeding in what Griffith had failed to achieve, while Alloway (1932, 1933) demonstrated transformation *in vitro* using cell-free extract of pneumococci. Upon his return to Rockefeller after a sick leave, Avery was made aware that his colleagues had confirmed Griffith's results and had even extended them. As is well known, after more than fifteen years of experimentation they then established that the transformation of one type of pneumococcus (avirulent) into another (virulent) was the result of the action of a deoxyribonucleic acid, or DNA, and not protein (Avery et al., 1944).[26] Avery's work underlined the need to bring molecular and chemical approaches to bear on the study of infectious diseases and, according to Nobel Prize laureate Frank Macfarlane Burnet (1899–1985), it signposted a broad shift from the study of infectious diseases to molecular biology (Burnet, 1968; see Amsterdamska, 1993; Mendelsohn, 2002). Transformation was later experimentally demonstrated to occur in the respiratory tract (Conant and Sawyer, 1967) and although further works on transformation continued as biochemistry and molecular biology developed in the 1960s the medical domain largely ignored the phenomenon of transformation discovered by Griffith (Downie, 1972).

The Accusation of Lamarckism

Early molecular biologists often saw as uninterrupted the line of intellectual descent going from Karl Nägeli (1817–1891) to Fred Griffith, and interpreted bacteriology, by and large, as "one of the last strongholds of Lamarckism" (Luria, 1947, p. 1; Olby, 1974; see also Morange, 1998). This is partly due to the fact that bacteriologists often described bacteria's adaptation to their milieu in terms of acquired traits. "Transformation of pneumococcus strains, as observed by Frederick Griffith," Angela Creager

[26] On the reception of Avery's paper see Stegenga (2011), Cresto (2008), Deichmann (2004), Morange (1998), and Olby (1974/1994). On the confirmation of Avery's results see Olby (1974/1994, Chap. 14).

noted, "was commonly viewed as evidence of Lamarckian inheritance" (Creager, 2007, p. 173). According to Olby, for example, Griffith had a "*Lamarckian scheme* of intraspecific variation in his mind," and he "was not surprised by the discovery of the transformation of types" (Letter from Olby to Lederberg, November 8th, 1972; emphasis added). "After having established that R cells derived from Type 1 could be transformed into S cells of Type II," Dubos also concluded that "Griffith suggested a *Lamarckian explanation* of the phenomenon" (1976, p. 135; emphasis added). "Strangely," Thomas Brock observed fifteen years later, "Griffith did not see his third (somewhat *Lamarckian*) hypothesis, interchangeability of type, as particularly radical" (1990, p. 218; emphasis added).

In the post-face of his book, Olby maintains that "the neo-Lamarckian account" he gave "is important for our understanding of the whole transformation story" (Olby, 1994, p. 456). But what does it mean to claim that Griffith's interpretation of his result was "Lamarckian," and why does it matter? For one thing, the Lamarckian reading of the transforming experiment was important because historians used it to explain scientists' initial reluctance in accepting Griffith's results. According to Olby, for instance, "Griffith's reputation was high, but his 'Lamarckian' ideas and his vague talk of a 'pabulum' could hardly have appealed to Avery who […] was at least committed to strictly chemical explanations" (1974, p. 178). Others, however, refrained from attributing the label of Lamarckism to Griffith. In reading Olby's manuscript for his book on the history of molecular biology, Lederberg felt that the author was trying to "force a theoretical outlook" on Griffith's interpretation of his own results. "It seems inappropriate," Lederberg wrote in response to Olby, "to put the label Lamarckian in a point of view that was not well informed about the nuances of the issue that it presents nor about the elements of related controversy in other fields of biology" (Letter from Lederberg to Olby, October 25th, 1972). Lederberg also said he would "hesitate to call Griffith a Lamarckian for this would imply some greater clarity […] than I believe he exhibited in his earlier paper" (Letter from Lederberg to Olby, October 25th 1972).

While it is easy to go from "transformation" to "transformism" to "Lamarckism," bacterial transformation bears little in common with what became known as the inheritance of acquired characters, typically (and often misleadingly) associated with the name of Lamarck – but that Darwin and several other biologists of the nineteenth and early twentieth century also accepted (for a recent study on neo-Lamarckism, see Loison, 2011). According to Lamarck, for example, variations always go from the less complex to the more complex (Jacob, 1973, p.

145). This scheme does not apply to Griffith's work that explored how bacteria could both lose and gain in antigenic complexity. Griffith's paper, furthermore, is rather silent about the fact that induced modifications via transformation are *heritable* (Downie, 1972; McCarty, 1985). This is not surprising, though, given that most bacteriologists of the 1920s "were unconcerned with whether bacterial variation was hereditary" an issue that seem far off from their practical concerns (Creager, 2007, p. 163; see Amsterdamska, 1987). Furthermore, scientists working in the 1920s did not sharply distinguish the concepts (nor the cause) of mutation and adaptation as we do today; but the most important point is that Lamarckism and Darwinism did not yet stand out as competing explanatory schemes – at least in bacteriology and microbiology – when Griffith discovered transformation: the genetic (and selectionnist) interpretation of transformation would take shape only during the 1940s within the neo-Darwinian framework (Creager, 2007). If one truly wants to relate Griffith to the transformist tradition in microbiology, one should probably look into Nägeli's microbiological works rather than in Lamarck's.[27]

After the Transforming Experiment: Griffith's Work on *Streptococcus pyogenes* and the Decline of Rheumatic and Scarlet Fever in Britain

Next to his research on pneumococcus and meningococcus, Griffith has been involved in a series of epidemiological and bacteriological projects investigating the etiology of rheumatic fever and streptococcal infections. Though its virulence was progressively declining since the late nineteenth century (Swedlund and Donta, 2003), rheumatic fever was still a serious cause of morbidity and mortality in the first three decades of the twentieth century. In the interwar period, researchers attempted to correlate the presence of a specific microorganism with the disease in order to devise an antisera-based treatment similar to those developed for pneumococcal diseases. Identifying the causative organism through slide agglutination was not straightforward, however. What germs and under what conditions they were normally found in humans was

[27] Griffith, indeed, did not place rigid limitations on the changes in serological types, as he considered that the emergence of one type rather than another depends more fundamentally on the material being provided, not on the species. He noted that, for instance, "in its *ultimate* form" the R pneumococcus "is the same, no matter from what type it is derived." The R pneumococcus posses antigens of both Types I and II and "could develop either S form according to the available material" (1928, p. 153; emphasis added).

problematic. The situation was also complicated due to the fact that precise classifications of bacteria (pathogenic and non-pathogenic) were lacking (English, 1989), which is what Griffith sought to remedy.

In the early 1920s, attempts at isolating the bacteria responsible for the disease had thus largely failed although the conviction that cases of rheumatic fevers were due to members of the streptococcus family remained widely held. As historian Peter C. English has described, several research strategies devoted to identifying the crucial factor(s) in the etiology of rheumatic fever emerged in the interwar period, including allergy, heredity, environment, and infection with group A hemolytic streptococcus (2002, p. 89). Griffith's study on group A streptococcus (GAS) was a turning point in the epidemiology of GAS and rheumatic fever in the twentieth century. He pursued this research, forming the last part of his written work, sometimes alone but often also alongside other scientific investigators who made decisive contributions to the elucidation of the bacteriological or to the epidemiological aspect of the disease in England and in the United States. These advances, in turn, allowed scientists to trace the sources of infection with greater precision and accuracy, prior to the advent of antibiotics.

Scarlet Fever

Beginning in 1926, Griffith studied hemolytic streptococci of human origin in relation to scarlet fever, one of the outcomes of streptococcal infections. Classification of types, as always, was a prerequisite to "more intimate knowledge of the interrelationship between the various forms of streptococcal infections" (Griffith, 1927, p. 372). The variety of bacteria was so great that without a proper way of sorting pathogenic from non-pathogenic form, attempts to locate the sources of infection were largely improbable. Using slide agglutination technique, Griffith demonstrated that the family of streptococcus comprises four main serological types and one heterogeneous group (*Ibid.*). Together with British bacteriologist William Gunn, he conducted an extensive and intensive study of one hundred cases of scarlet fever in Britain, mostly from schools, in a combined bacteriological and clinical perspective. In their paper, Gunn and Griffith emphasized the correlation between types of streptococcus, the level of toxin production, and the severity of scarlet fever infections (Gunn and Griffith, 1926). For half of the cases, the same serological type was found throughout the hospital treatment of patients. For the other half, however, there was an "apparent change of type" during the course of the disease (Gunn and Griffith, 1926, p. 253). Gunn and Griffith proposed three hypotheses: the serological

types were too instable to warrant a useful classification; the bacterium may be stable outside the body but was liable to change in contact with antibodies formed to resist infection; reinfection occurred as the patients were close to one another. In contrast with the other paper published in 1928, Griffith did not decide for type-transformation: ''While the possibility of modification of type cannot be excluded,'' they settled for the last hypothesis: reinfection (Gunn and Griffith, 1926, p. 253).

Rheumatic Fever

British epidemiologist J. Alison Glover (1874–1963), author of the droplet infection theory of transmission of rheumatic fever, was one of the main scientists to document the decline of this disease in Britain during the 1930s and 1940s. In his *Milroy Lectures*, Glover concluded that rheumatic fever was becoming an ''obsolescent disease,'' apart for children (Glover, 1930). Together with Griffith, he investigated several cases of tonsillitis outbreaks in relation to scarlet fever in boarding schools in England in the early 1930s (Glover and Griffith, 1930a, b; Glover and Griffith, 1931). In a paper on the sequel of tonsillitis, they established that scarlet fever is only one of the possible outcomes of hemolytic streptococcal infections, though streptococci were unified as a species. This conclusion was put forward thanks to Griffith's method of serological analysis that allowed classifying the chief types involved in outbreaks of streptococcal infections under study. This method also helped clarifying the epidemiological importance of types as well as their actual association with clinical conditions. Their results and public health recommendations appeared in the pages of the *Lancet* and the *British Medical Journal*.

Griffith's fundamental paper related to rheumatic and scarlet fever was published in 1934 in the *Journal of Hygiene*. There, he exposed how he patiently sorted the species *Streptococcus pyogenes* into 27 distinct serological types. He noted that like pneumococcus, streptococcus had become differentiated into a number of ''serological races'' with different pathological and epidemiological values though they continued to form a ''well-defined bacterial species'' (Griffith, 1934, p. 578). What was the motivation behind this large-scale project carried out by slide agglutination technique? In his 1972 note to Lederberg, Coburn remarked that one important ''conceptual antecedent'' to Griffith's ''colossal task of identifying hemolytic streptococcus serologically'' was the identification of variants that had great ''infectivity.'' For Griffith, typing might provide valuable insights about the transformation of harmless saprophytes into virulent pathogens.

> There is no doubt in my mind that Griffith, the bachelor, was inspired by the *romance of a concept*, i.e. one variant of *Streptococcus pyogenes* with great 'infectivity' (commonly called virulence) for man could produce a devastating epidemic. And believe me when one is inspired with this concept all other matters seem momentarily insignificant (Letter from Coburn to Lederberg September 28th 1972, p. 2, emphasis added).[28]

As usual, Griffith thought that a better differentiation of bacterial strains should come first. ''The importance in epidemiological studies of a reliable method of identification of a bacterial species,'' as he put it in his talk at the 2nd International Congress in Microbiology, ''is unquestionable'' (1937, p. 132). Aligned with his prior interest in finding how a common saprophyte may be able to propagate an epidemic under appropriate conditions or how a virulent type was reverting to a rather inoffensive one, as for instance during convalescence, Griffith tried to pin down the multiple causes of changes in virulence. In the conclusion of his 1934 lengthy paper, he related the loss of severity in scarlet fever to a change in the ''serological constitution'' of the population.

> The evolution of serological types in nature is an interesting subject for speculation. It seems probable from the severity of scarlatinal epidemics in the past was originally much more virulent and toxigenic [...] In consequence of experience of streptococcal infection being more common in crowded urban communities man has developed a considerable degree of herd immunity, and it may be that the resistance which the streptococcus has had to contend with has resulted in the development of the existing multiplicity of serological races. Each of these races or types differs, however slightly, from another in infectivity, invasiveness and toxigenic capacity in virtue of its individual antigenic constitution (Griffith, 1934, p. 582).

Epidemics of scarlet fever were much more devastating in the late nineteenth century, as Glover and others had demonstrated, and as clinicians had also observed (English, 2002). For Griffith, however, the decline in virulence was not only due to the global increased in resistance on the part of the population (''herd immunity'') but also the result of the germ's loss in infective capacity. Griffith derived a lesson of

[28] Topley offered a similar view about Griffith: ''Many field epidemiologists, and in particularly the late Dr. Fred Griffith, have been convinced that the observed behavior of certain human infections, such as those due to haemolytic streptococci, demand the hypothesis of the existence of special epidemic strains of the parasite concerned, with heightened power of producing disease by contact infection'' (Topley, 1941, p. 352).

epidemiological significance from this two-way process, very much along the lines of his paper on transformation: the "spread of disease in a community," he argued, "means ultimately not only increased resistance on the part of the host but also alteration and attenuation of the parasite" (1934, p. 583). According to Griffith, a susceptible human population and a pathogen that was once very virulent were locked in a long-term process of adaptation that might lead to a harmless relation. Indeed, the English bacteriologist claimed that while most epidemics of scarlet fever in Britain were caused by type I and II, some types were increasingly causing tonsillitis without rashes meaning that serological types can "lose their toxigenicity" (1934, p. 583). "If the present tendency is maintained," Griffith continued, scarlet fever "will disappear as a clinical entity" (*Ibid.*). In sum, there was a process towards decreased levels of virulence as humans' immunity rose and the streptococcus's pathogenic power declined over time. Did Griffith consider the described changes in bacterial virulence to be the result of physiological adaptation of the bacteria or of a selection of less virulent variants? Given his constant emphasis on changes in the biology of the parasites to account for the spread of diseases, it is likely that such physiological explanation might have appeal to him, perhaps even more than a purely selectionnist hypothesis sorting out strains that varied in virulence power (on the distinction between adaptation and selection, see Creager, 2007).

Questions of Methods: The Correspondence Between Griffith and Lancefield – 1935–1937

In the early 1930s, two rival methods existed for identifying pneumococcal strains (Parker, 2001; see Lancefield 1933): Griffith's type agglutination technique and Lancefield's precipitin test reactions (where strains were identified on the basis of C-carbohydrates and M protein). Correspondence between Griffith and Lancefield located at the Rockefeller Institute in New York bring additional light on the methodological issues arising in the classification of pneumococcus during that period. It was the possibility of classifying *Streptococcus pyogenes* into 27 distinct types as proposed by Griffith led to a letter of interest by Rebecca Lancefield to the British bacteriologist in 1935.

> Dear Doctor Griffith, I have just read your paper in the current *Journal of Hygiene* with the greatest interest. I should not have supposed it is possible to classify the majority of strains of *S.*

> *pyogenes* into so small a number as 27. It certainly makes a much more workable situation in this group if one can do that (Letter from Lancefield to Griffith, January 22nd 1935).[29]

Griffith responded quickly and positively to Lancefield's letter, who turned out to be right in the end – there were more than 27 strains. Between 1935 and 1937 the two bacteriologists corresponded frequently. Most of their letters bore on the methods of typing pneumococci. The letters do not mention, however, whether they met in person during the International Congress in Microbiology in London in 1936 to which both participated.

Lancefield and Griffith were keen to know whether their methods were compatible in typing streptococcus stains. ''I have not finished checking some of our strains against your sera yet,'' wrote Lancefield, ''but enough is done to show the general agreement between these two methods of typing Group A strains'' (Letter from Lancefield to Griffith August 14th 1935, p. 1).[30] This was a reassuring finding as scientists had been wondering whether the two methods would indeed yield the same result: ''It seems to me'' Lancefield continued, ''that wherever I have had good anti-M sera for making the comparison, the results of typing by these different techniques agreed very well. This was, of course, what I expected, but so many people have asked me whether our method would give the same results that I am very glad to have definite information on the subject'' (Letter from Lancefield to Griffith, August 14th 1935, p. 1).[31] To achieve uniformity, Lancefield also adopted the numerical classification proposed by Griffith (McCarty, 1987).

Despite general agreement in typing, discrepancy arose sometimes between their different methods, and they endeavored to account for it: ''Our results then with these strains are opposed ''Griffith pointed out,'' and it is necessary to seek some explanation of this divergence'' (Letter from Griffith to Lancefield, April 29th 1937).[32] Though they had deep respect for one another Griffith and Lancefield ''did not see eye to eye on methodology'' and ''were never converted to each other's approaches'' (McCarty, 1987, p. 235). The exchange of letters between the two bacteriologists, interrupted by Griffith's death, however, shows that despite his skepticism to ''new claims in streptococcal work'' he was beginning to acccept other bacter-

[29] Letter from Rebecca Lancefield to Fred Griffith, January 22nd, 1935, Rebecca Lancefield papers, Rockefeller University, Series 450, L221, Box 3, folder 1, p. 1.

[30] Letter from Rebecca Lancefield to Fred Griffith, August 14th 1935, Rebecca Lancefield papers, Rockefeller University, Series 450, L 221, box 3, folder 1, p. 1.

[31] *Ibid.*, p. 3.

[32] Letter from Fred Griffith to Rebecca Lancefield, April 29th, Rebecca Lancefield papers, Rockefeller University, Series 450, L 221, box 3, folder 1.

iological systems of classification that were then commonly in use for many years (Letter from Maxted to Lederberg, no date).

Conclusion

Fred Griffith was a medical bacteriologist and public health officer by training whose work was deeply rooted in epidemiological considerations, as he was acutely interested in the biological study of host–parasite relations and their consequences for diseases at the population level. Questions of epidemiology, in fact, fundamentally drove his bacteriological work rather than the other way round. Epidemiology broadly understood provided him with an interface between biological and medical understanding of host–parasites relations. Interested in unraveling the emerging patterns as well as the sources of infections, most of his reports attempted to relate bacteriological knowledge with the epidemiological path of the disease under study. As Topley once put it, Griffith was a "field epidemiologist" (1941, p. 352). Griffith's approach differed from the "experimental epidemiology" defended by Greenwood and Topley at the School of Tropical Medicine and Hygiene in London, however. In contrast with mathematical approaches to epidemiology that would be prevalent after the Second World War, and would become the hallmark of modern epidemiological practice (Amsterdamska, 2005), Griffith did not draw on statistical methods or mathematical tools. What he did was generate detailed tables in which he meticulously reported the results of his numerous experiments with animal models into which he inoculated various bacterial strains. In this respect, he was not an epidemiologist working with "modern" mathematical tools but rather a medical bacteriologist of the "old school" that was still prevalent in the interwar period.

The serum type agglutination technique Griffith developed on a large-scale was devoted to advance a more precise differentiation of microorganisms and to the production of serum therapy and vaccines. The differentiation of hemolytic streptococci into types he accomplished was likely one of his most valuable scientific contributions though it is most of the time ignored by commentators, who concentrated almost exclusively on the transformation study. This work on classification, continued and broadened by Lancefield's method after Griffith's death, permitted scientists and public health officers to identify the sources of infection for deadly diseases such as scarlet fever, rheumatic fever, sore throats, puerperal fever as well as wound infections and to develop preventive and prophylactic measures

(Hare, 1940). After the introduction of penicillin and sulfa drugs in the early 1940s, serum therapy became soon obsolete – although this technique recently surfaced in the context of the Ebola epidemics in West Africa to cure an American doctor infected with the virus.

Despite his practical achievement with typing hemolytic streptococci, Griffith's name became closely associated with the phenomenon of transformation. Considering Griffith's earlier studies has helped revisiting long-established historical narratives and has permitted unraveling some of the "conceptual antecedents" of the transforming experiment Lederberg was after. First, Griffith's classification of serological races was informed by evolutionary thinking about the variation in types, although his understanding of the phenomenon remained intellectually closer to natural history than to modern bacterial and molecular genetics. For instance, Griffith was less interested in the heritable dimension of the R to S changes he experimentally provoked than in their current adaptive effect and their wider epidemiological significance. Griffith, furthermore, was open to the possibility of witnessing changes in serological types several years *before* his studies on the R and S forms published in 1923. Yet if Griffith was "prepared" to observe transformation in 1928, as Olby pointed out, it was not, however, because he was committed to neo-Lamarckian explanations but rather because he had an *evolutionary* scheme of biological variation in his mind. The indeterminacy of terms such as "variation," "transmutation," and even "adaptation," as employed by Griffith and other bacteriologists and microbiologists in the 1920s and 1930s, together with his lack of emphasis on intergenerational change, prevent a strict "Lamarckian" interpretation of his results.[33]

Second, in contrast with most accounts of Griffith's achievements, we have seen that the English bacteriologist returned to the topic of transformation one year after his 1928 study and discussed other works in relation to it. Whereas some bacteriologists claimed that microbial strains are very stable, already in 1911, while he was an investigator during the Royal Commission on Tuberculosis, Griffith considered the possibility of "transmutation" of one type of bacteria into another. This finding extends Robert Olby's suggestion that Griffith's discovery of transformation was perhaps "quite *deliberate*" (1994, p. 446; emphasis in original). His finding that one strain of pneumococcus can revert into another of a different type, in retrospect was not a mere accidental

[33] It would be interesting to contrast Griffith's view of "adaptation" with the one developed by French microbiologist Charles Nicolle (1866–1936), laureate of the Nobel Prize in 1928 and pioneer of ecological approaches in medicine.

observation, but a direct continuation of his earlier ideas on the possibility of transmutability of bacteria. A consequence of taking into consideration Griffith's career as a bacteriologist interested in the origin and disappearance of infectious diseases is that transformation does not come across as a "quirky medical idea" but as part of a broader research tradition – starting with Pasteur in the second half of then nineteenth century – aimed at understanding the coming and going of infectious diseases, the changes in virulence, and the nature and scope of biological variation more generally (Mendelsohn, 2002).

Thirdly, it is worth mentioning that Griffith's working methods, located at the border between the bacteriological laboratory, field experiments, and epidemiology are reminiscent of the meticulous works of comparative pathologist Theobald Smith who investigated diseases such as tuberculosis and Texas fever at the turn of the past century in the United States. Not unlike Smith, Griffith attempted to study host–parasite relations and infectious diseases not only at the "lab-field border" (Kohler, 2002) but from all points of view; he wanted to understand the details of the biology of host and parasites precisely, although he did not believe that "exhaustion of susceptible materials" was sufficient to account for the decline of an outbreak. As to the effects of a prolonged relation between a host and a pathogen, Griffith's discussion in the 1934 paper illustrates the evolutionary underpinning of his views on the matter. In the 1960s, when molecular biologists applied their tools to unpack the biology of the streptococcus organism responsible for rheumatic and scarlet fever, clinicians and bacteriologists had long documented their decline. The long history of scarlet fever to which Griffith explained some central epidemiological features provides finally an illustration of Theobald Smith's perspective on which harmlessness on both sides is the expected outcome of long-term biological associations – a position endorsed by several pioneers of the ecological approach to disease far into the second half of the twentieth century (Méthot, 2012).

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