Pneumococcal Transformation – A Backward View

Fourth Griffith Memorial Lecture

By A. W. DOWNIE

Department of Medical Microbiology,
University of Liverpool

(Delivered at the General Meeting of the Society for
General Microbiology on 11 *April* 1972)

> *Professor C. M. MacLeod, President of the Oklahoma Medical Research*
> *Foundation, Oklahoma, U.S.A., who had agreed to give the* 1972 *Griffith*
> *Memorial Lecture, died on* 12 *February,* 1972. *No text of his lecture was*
> *available. Professor A. W. Downie generously agreed, at short notice, to*
> *give the lecture which follows, and which uses Professor MacLeod's intended*
> *title.*

Professor Colin MacLeod who was to have given this memorial Fred Griffith lecture had very obvious qualifications for his selection. He had worked in Avery's laboratory at the Rockefeller Institute for Medical Research for eight years and was joint author of the original paper in which it was shown that DNA was responsible for transformation of pneumococcal types, the phenomenon first described by Griffith. Moreover he continued and extended the work on transformation for some years afterwards at the New York University College of Medicine where he had been appointed Professor of Bacteriology at the early age of 32. I cannot pretend to have the knowledge and experience of MacLeod in this field, but, because I knew personally the workers concerned in at least the early stages of the transformation story, I decided to talk on the subject that Dr MacLeod had chosen – although the view that I shall present is likely to be more backward than he had intended. Then I had not recently read the first and third Griffith memorial lectures by Professor Hayes (1966) and Professor Pollock (1970). Professor Hayes interpreted Griffith's experiments in terms of present knowledge of bacterial genetics and Professor Pollock's scholarly history of the discovery of DNA emphasized the significance of the work on transformation for the development of molecular biology. I shall not attempt a discourse on such a high scientific level.

A few days ago I received some notes from Mrs MacLeod which showed that Colin MacLeod had intended to describe in detail the laborious work by Avery, himself and McCarty lasting over several years, which finally led to the conclusion that DNA was the transforming principle which conveyed the inheritable characters of one pneumococcal type to another. This would have been an account of absorbing interest which McCarty may one day relate. But because of lack of inside information I cannot follow this line. Instead I propose to say something about the way Fred Griffith's work on transformation arose out of his predominant interest in bacterial typing in relation to epidemiology, and then to say something of Avery, whose life-long interest in the pneumococcus made possible the elaboration of Griffith's discovery, and for whose work Griffith had the greatest admiration.

Fred Griffith was born in Cheshire. After a distinguished undergraduate career he graduated in medicine at Liverpool in 1901. He had a fellowship in the Department of Pathology and then, after some work on the tubercle bacillus with his brother A. S. Griffith, he joined the staff of the Local Government Board. When the Local Government Board laboratories were taken over by the Ministry of Health during the First World War, Griffith and Scott moved to Dudley House in Endell Street. There the Pathological Laboratory of the Ministry of Health functioned until the outbreak of World War II, when its functions were taken over and expanded into the Emergency Public Health Laboratory Service (E.P.H.L.S.). When I first visited the laboratories in the early 1930s they consisted of one office, a reasonably large laboratory which Griffith shared with Scott, and a media kitchen where two technicians worked. The lower two floors of Dudley House were occupied by the Post Office. Griffith and Scott did all their own bench work with very little up-to-date equipment even for that period. Nevertheless excellent work was done, and both Griffith and Scott gave endless help to bacteriologists investigating infectious disease in various parts of the country. As Hedley Wright (1941) wrote of them, 'A foreigner, visiting for the first time the laboratory of the Ministry of Health in London, must have been little short of appalled to see how meanly this fundamental activity of a wealthy country was housed and must have wondered how, thus cabined and confined, these two world-famed workers managed to exist, let alone function, in such a chaotic environment. But he would not be long there before he knew that they did so because they were Scott and Griffith, who could do more with a kerosene tin and a primus stove than most men could do with a Palace.' They shared the same laboratory for most of their working lives and with Allison, who joined them in the early '30s, they formed the nucleus of staff around which the E.P.H.L.S. was built. They died together when Griffith's London flat, where Scott was staying at the time, received a direct hit during an air raid in February 1941, a few weeks after Scott had succeeded Topley as Director of the Emergency Public Health Laboratory Service.

Griffith's main scientific interests were related to the epidemiology of infectious disease. He felt that more had to be done to identify conclusively the types and the species of bacteria found in various outbreaks, if their epidemiology was to be understood and effective control measures taken. Hence his interest in the typing of tubercle bacilli, meningococci, pneumococci and streptococci. The discovery of immunologically distinct types of pneumococci was made by Neufeld & Handel (1909, 1912). Like other bacteriologists earlier this century – long before sulphonamides or antibiotics were discovered – they made attempts to prepare immune sera which might be of value in treating severe infections such as lobar pneumonia, a more common and more dreaded disease then than it is today. The serum first prepared by Neufeld and Handel would protect mice against some strains of pneumococci from pneumonia cases but not others, and they found that there were at least three immunological types. These results were confirmed by Dochez and Gillespie in 1913; and in the Rockefeller monograph published in 1917 by Avery, Chickering, Cole and Dochez the examination of strains from cases of pneumonia led to the definition of types I, II and III with a heterogeneous Group IV. Griffith in 1922 found roughly the same distribution of types of pneumococci in pneumonia cases in London, as the American workers had noted in New York. Among the Group IV strains Griffith established that there were 12 new serological types and certainly many more; for, of 77 group IV London strains examined, 28 remained which did not fall within the 12 new types. However, the finding of Group IV strains of low virulence from individual patients, usually during convalescence, suggested to Griffith that either (a) the virulent type I, II or III strains

might simply have been replaced by an avirulent strain as might be found in normal persons, or (*b*) the immune response of the host had degraded the virulent strains into untypable variants. This latter possibility set Griffith working with rough non-capsulated avirulent pneumococci. These he had produced in the laboratory by growing smooth virulent type I, II or III strains in the presence of homologous type antiserum, a technique which had been used by Stryker in Avery's laboratory some years before (Stryker, 1916). The possibility of acquisition of virulence by these experimentally produced rough forms, and the possible reversion to virulence of weakly virulent strains found in the throats of convalescent patients and healthy persons, prompted the experiments which resulted in the discovery of transformation of types. The subcutaneous injection of large doses of rough pneumococci into mice occasionally led to fatal infections from which smooth virulent pneumoccoci of the original type were isolated. Reversion to virulence could be achieved more readily if rough pneumococci, derived from type II, were injected together with a large dose of heat-killed organisms of smooth virulent type II. The most surprising result, however, came from Griffith's control experiment, where heat-killed smooth organisms from type I were injected along with rough organisms derived from type II. The mice died of generalized infection with virulent type I pneumococci; an apparent transformation of pneumococcal type. Griffith found this result, an example of pure serendipity as Pollock pointed out, difficult to believe, and as his paper of 1928, republished in the *Journal of Hygiene* in 1966, shows, he made many experiments with extensive controls to establish this surprising transformation of one pneumococcal type into another. Because Griffith was conditioned to believe that bacteria existed in immutable types, offering a solid basis for epidemiological investigation, one can imagine he was at first loath to accept his own results. Fred Griffith was a very shy person, rarely went to meetings and could not readily be persuaded to read a paper. I think it is safe to say that if he had been persuaded to communicate his findings to the Pathological Society – there was no Society of General Microbiology then – those who knew Griffith's work would have found his results surprising, but would have accepted them. It was a long time after his experiments were completed before the results were published, and by then he had started his studies on streptococcal infection. According to Scott, Neufeld had visited their laboratory and had been told of Griffith's transformation experiments. Some months after he had returned to Berlin, Neufeld wrote to ask Griffith when the results were to be published, as he, Neufeld, had confirmed Griffith's results and wished to write them up. This explains why Neufeld and Levinthal's confirmatory paper appeared so soon after Griffith's; and indeed, in Neufeld's paper it is stated that one of the authors had visited Griffith's laboratory some months before, and been told of Griffith's results and the technique used (Neufeld & Levinthal, 1928).

Most of the subsequent work on transformation was done in Avery's laboratory at the Rockefeller Hospital in New York, which had been the centre of pneumococcus research in America for the previous fifteen years. But the initial confirmatory experiments were not made by Avery, who for many months refused to accept the validity of transformation and was inclined to regard the finding as due to inadequate experimental controls! This scepticism was understandable in one who had devoted so much effort and skill to the doctrine of immunological specificity. But Avery was suffering at that time from thyrotoxicosis and left the laboratory for some months. During that time his colleague Dawson not only confirmed Griffith's findings, but went further and showed that transformation of type II to type III could be effected *in vitro* by incubating a very small inoculum of living R cells, derived from type II, together with anti-R serum and a concentrated suspension

of heat-killed virulent type III (Dawson & Sia, 1931). He confirmed Griffith's observation that transformation did not occur if the smooth type III suspension was heated above 80 °C. He further found that heat-killed preparations from old cultures were useless, and that freezing and thawing of previously effective preparations destroyed the transforming principle (Sia & Dawson, 1931). He suggested that the essential factor was destroyed by bacterial enzymes. Pure S.S.S. – that is, type-specific capsular polysaccharide from type III – failed to transform rough forms derived from type II. Later he found that even smooth type II could be transformed into type III, apparently without the intermediary of rough forms (Dawson & Warbasse, 1931). The next step was taken by Alloway, also working in Avery's laboratory (Alloway, 1932, 1933). He found that transformation could be achieved by bacteria-free extracts alone prepared from young cultures of virulent pneumococci. The sedimented bacteria were dissolved by sodium desoxycholate and heated at 60 °C for 10 minutes. The extracts could be filtered through Berkefeld candles, and absorbed with charcoal, without losing activity. The transforming principle could be precipitated by 10 vol. of alcohol or acetone. The activity of the final preparation was such that 0·05 ml of extract (50 ml concentrated from 5 l of culture) was sufficient to produce transformation of R forms in the presence of serum or serous fluid. These extracts contained specific polysaccharide in sufficient concentration to induce active immunity in mice. However, Alloway did not think that specific polysaccharide was the transforming principle and concluded that 'if S.S.S. is involved it is present in a different physical state, or in combination with some other substance which confers on it properties not found in the purified substance'.

No further significant paper on transformation appeared until the famous paper in 1944 by Avery, MacLeod and McCarty. But this paper was the result of several years' labour, as is obvious from the paper itself and from the letter, quoted by Pollock, written by Avery to his brother Roy in 1943. In 1933 Alloway left Avery's laboratory and Dawson had by then gone to the Presbyterian Hospital in New York. Neither apparently continued the work on the transforming principle. At this stage nothing was definitely known about the nature of the active substance. Was it S.S.S. combined perhaps with some other substance as Alloway had suggested, was it protein, or was it something else?

When MacLeod came to Avery's laboratory as a junior member of staff in 1934 he took up the study where Alloway left off in 1933. From the start he had difficulty in obtaining regularly active extracts, and it took another three years to work out the conditions necessary for constantly reproducible results. Not all rough strains of pneumococci were suitable for transformation – we would use the term 'competent' today. This difficulty was overcome by transferring a smooth type II through 36 cultures in the presence of type II serum and plating out the 36th subculture. Amongst the rough single colony isolates one – R36A – gave a high and consistent yield of transformants in the presence of heat-killed type III cells, and was used in all subsequent work. When it came to preparing active transforming extracts from virulent type III pneumococci, unexpected difficulties were met. Not all batches of broth proved suitable, but it was found that absorption of the broth with charcoal made most samples satisfactory. Enzymes from the extracted pneumococci slowly destroyed the transforming principle, and some samples of serum added to the reaction mixture had the same effect. These difficulties were overcome by heating the pneumococcal suspension before extraction, and the serum, to 60 °C for 30 minutes. The active principle had been found by Alloway to be precipitated by alcohol, and this step was regularly adopted because stability was thereby improved. Not until 1937 could active transforming extracts be consistently prepared and a method of titrating this activity be developed. Then the work on

purification began, for up to this point the transforming extracts contained, among other things, pneumococcal protein, non-specific polysaccharide (C substance) and type-specific capsular polysaccharide. The protein could be removed by Sevag's method of repeated shaking with chloroform without affecting the concentration of transforming principle. The type III specific polysaccharide could be removed by digestion with Dubos's specific enzyme, which I will mention later. The active principle could be separated out from the resulting fluid by careful addition of alcohol, and was further purified by repeated solution and reprecipitation. By chemical analysis, by its ultraviolet absorption curve and its behaviour with various enzymes in comparison with a pure preparation of desoxyribonucleic acid, the active principle was identified as DNA. The authors, however, were guarded in their summary and conclusions; for example: 'Evidence is presented, that the chemically induced alterations in cellular structure and function are predictable, type specific and transmissible in series'; and 'The evidence presented supports the belief that a nucleic acid of the desoxyribose type is the fundamental unit of the transforming principle of Pneumococcus type III'.

Avery published only two further papers on the nature of the transforming principle, both with McCarty (McCarty & Avery, 1946). McCarty had prepared a relatively pure desoxyribonuclease from ox pancreas and, with Avery, showed that this enzyme destroyed the transforming activity of the active principle under the same conditions that it broke down purified DNA from calf thymus. In the second paper McCarty and Avery showed that much higher yields of the transforming principle could be obtained by the use of citrate in the presence of magnesium salts to inactivate DNase in the pneumococcal suspension from which the transforming principle was to be prepared. These papers merely strengthened the case for DNA being the active principle, and met the objections of those who suggested that some protein contaminant was responsible for transformation, rather than DNA itself. Later Hotchkiss, who had come to Avery's department in 1937 and continued to work there after Avery left in 1947, provided further chemical evidence that the preparations of DNA responsible for transformation contained no contaminating protein. Although Avery himself never in print identified DNA as the essential genetic material of the pneumococcal cell, his pupils were more committed. In a paper published in 1949 on pneumococcal transformation Harriet Taylor (later H. Ephussi-Taylor), who was working in Avery's laboratory, wrote: 'It appears justified therefore to visualize the transforming principle much as the geneticist pictures genes' (Taylor, 1949).

McCarty turned to the study of streptococci soon after the two publications with Avery in 1946; but MacLeod, who had moved to University College of Medicine, New York, in the early 1940s continued work on pneumococcal transformation. In transformation experiments, Austrian and MacLeod showed that the type-specific M protein of pneumococcus, which they had discovered, could be transferred independently of the specific capsular polysaccharide (Austrian & MacLeod, 1949*a*, *b*). They made the important suggestion that transforming extracts of encapsulated pneumococci contained a multiplicity of deoxyribonucleic acids which controlled the specificity of the several cell characters described. In a paper published in 1950 with Krauss, MacLeod found that among experimentally transformed pneumococci derived from various serological types, the amount of S.S.S. formed and the virulence of the strain was controlled by the genetic apparatus of the donor strain (MacLeod & Krauss, 1950). During the next few years MacLeod extended the study of transformation reactions to streptococci. In an examination of streptococci from various Lancefield Groups and of viridans strains, he found two strains of viridans streptococci which could be transformed to streptomycin resistance by DNA-containing

extracts from a streptomycin-resistant pneumoccocus. Conversely, streptomycin-resistant variants of these two strains of streptococci were able to confer this property to a sensitive pneumococcus. Resistance to optochin could be transferred by naturally resistant strepto-cocci to the naturally sensitive pneumococcus. The two strains of viridans streptococci were more efficient receptors of pneumococcal transforming principle than were pneumo-cocci, and it was concluded that efficiency of transformation did not necessarily indicate closeness of relationship (Braco, Krauss, Roe & MacLeod, 1957). In further transformation reactions between these two streptococcal strains and a rough pneumococcus, using strepto-mycin resistance as a marker, he found that the receptor strain was more easily transformed when the donor strain was, itself, a transformant which had received DNA from the recipient species (Krauss & MacLeod, 1963). MacLeod's participation in the original discovery of DNA as the transforming principle, and his continued interest in bacterial genetics, explains the title that he had chosen for this memorial lecture.

To go back a little to the discovery of DNA as the transforming principle: it seems, as has been emphasized by Pollock and more recently by Wyatt (1972), that the fundamental importance of Avery's work was not generally appreciated for several years, perhaps not until Hershey and Chase's work with bacteriophage in 1953. But although Avery and his colleagues were rather careful in the conclusions in their paper, and left their options open, they clearly realized the significance of DNA as the genetic material of the bacterial cell. Sir Henry Dale, in his presidential address to the Royal Society in 1946, announced the award of the Copley Medal to Avery. Referring to the transformation paper of 1944 he said: 'Here surely is a change to which, if we were dealing with higher organisms, we should accord the status of a *genetic* variation; and the substance inducing it -- the gene in solution, one is tempted to call it – appears to be a nucleic acid of the desoxyribose type'. Burnet visited Avery in December 1943, just after the paper had gone to press, and heard at first hand the latest results on the transformation story. In a letter to his wife at this time he wrote: 'Avery has just made an extremely exciting discovery which, put rather crudely, is nothing less than the isolation of a pure gene in the form of desoxyribonucleic acid'. This was probably Avery's interpretation of his own work. Burnet goes on to com-ment, writing in 1968: 'nothing since has diminished the significance or importance of Avery's work'.

Avery and Griffith were in many ways rather similar characters. Both were confirmed bachelors. 'Both were extremely modest, meticulously careful in their experimental work and extremely generous with time spent helping others. Both were almost excessively cautious in reaching conclusions, and both made major contributions to knowledge relatively late in life' (Pollock, 1970). He might have added that both had brothers who were eminent bacteriologists and both had one particularly close friend throughout their working lives – Griffith had Scott and Avery had Dochez. But there was one great difference in their working conditions. Griffith was always a lone worker – perhaps only partly because funds were not available to pay for assistants. On the other hand, Avery, in the Rockefeller Hospital, always worked with a team and could, when necessary, enlist the assistance of those whose special knowledge or skills would help to solve the problems which then occupied his attention. For, although he was rather shy and avoided speaking at meetings whenever he could, in private conversations his enthusiasm for his work could be presented with dramatic force and persuasive eloquence.

In 1913 Avery was asked by Rufus Cole, then director of the hospital, to join the group working on lobar pneumonia. Dochez & Gillespie (1913) had just confirmed Neufeld's observations on the multiplicity of serological types of pneumococci. With Avery's arrival

at the hospital there began a collaboration with Dochez which persisted in an informal but highly creative form for years after Dochez left the Rockefeller Institute, to become a professor of medicine at Columbia. As Dubos wrote: 'The two men shared a bachelor's apartment for many years and apparently never tired of discussing, night after night, problems of aetiology and pathogenesis. It would demand much psychological perspicacity to delineate the contributions of each participant in these midnight dialogues which had so much influence on the evolution of medical microbiology' (Dubos, 1956). Avery's work with the Rockefeller team began with the study of pneumonia patients and the strains of pneumococci isolated from them. In 1917 Dochez and Avery published their first paper on the type-specific soluble substance (S.S.S.) of pneumococcus (Dochez & Avery, 1917). This substance appeared after a few hours in pneumococcal cultures. S.S.S., of the type corresponding to the infecting organisms, was detected in the urine and blood of a proportion of pneumonia patients, and its presence in the blood was a bad prognostic sign, since most of these patients died. It was to determine the chemical nature of S.S.S. that Avery persuaded Michael Heidelberger to join his department. Their work led to the isolation and characterization of the specific capsular polysaccharides of the first three types of pneumococci (Heidelberger & Avery, 1923, 1924; Heidelberger, Goebel & Avery, 1925). The purified preparations, first made, were non-antigenic when inoculated into rabbits, and were regarded as haptenes. But it was shown by Schiemann and his colleagues (1927, 1931) that active immunity to infection could be induced in mice by the injection of very small doses – concentrations below that which would precipitate with the corresponding type-specific serum – while larger doses had no immunizing effect. Indeed, large doses produce the state of specific immunological paralysis. The earlier preparations had been made from suspensions of pneumococci from young cultures, or from the fluid from eight-day cultures; their isolation had involved heating to 100 °C and the use of strong acid and alkali. Less drastic treatment subsequently yielded from cultures of type I better preparations – the acetylated form – which, in a dilution of 1 in several million, would immunize mice and which would absorb all antibodies, including protective antibodies, from type I immune serum (Avery & Goebel, 1933). Francis and Tillett, in Avery's department, showed that the specific polysaccharides were antigenic on intradermal injection into man and that the deacetylated product would also induce the formation of mouse-protective antibodies in normal persons (Francis & Tillett, 1930; Francis, 1934). This finding later led to the use of purified specific polysaccharides in place of whole pneumococcal vaccines in prophylactic immunization of susceptible populations against pneumococcal infections. MacLeod was actively concerned with such prophylactic trials in military populations during the war.

By 1930 it had been established, largely through the work of Avery and his group, that the virulence of pneumococci was dependent on the capsular polysaccharide and that immunity, type-specific in character, was dependent on antibodies to it. It occurred to Avery that, if some means could be found of breaking down the capsular polysaccharide of infecting pneumococci in the animal body, the offensive mechanism of the organism would be lost and the animal might be cured. He thought that in soil, where complex carbohydrates must be broken down in the decay of vegetable matter, micro-organisms might exist which would break down the specific pneumococcal polysaccharides. At this time Dubos, who had been working in Waksman's laboratory in New Jersey, came to visit Avery. When Avery learnt that Dubos had been interested in soil microbiology, he immediately enlisted his assistance to tackle the problem he had in mind. A medium containing only ammonium sulphate, dibasic potassium phosphate and type III capsular polysac-

charide was used. Bacteria from samples of soil served as inoculum. Decomposition of the polysaccharide in the medium was tested at intervals by precipitation tests with type III antiserum. From one soil sample the bacteria were found to break down the polysaccharide. After months of subculture and purification by dilution, this decomposition was found to be due to an aerobic Gram-negative sporing bacillus. Filtrates of cultures of this organism were highly specific for type III polysaccharide and had no effect on polysaccharides from other pneumococci (Dubos & Avery, 1931). Enzyme preparations were found capable of curing mice infected with type III pneumococci (Avery & Dubos, 1931), rabbits infected intradermally with a virulent type III pneumococcus (Goodner, Dubos & Avery, 1932) and, finally, had a curative effect on pneumococcus type III pneumonia in cynomolgus monkeys (Francis, Terrell, Dubos & Avery, 1934). The potency of different lots of the enzyme varied, but even after more purified preparations were obtained (Dubos, 1935) Avery was cautious – his younger assistants thought too cautious – about testing the value of the enzyme preparations on type III pneumonia in man. The discovery of sulphonamides at this time obviated the need for such tests, and satisfactory trials of the enzyme were never made in pneumonia patients. Thus a brilliant idea, which led to a great deal of interesting and successful experiment, never led to the clinical application which had been hoped for.

The discovery of the biological specificity and antigenicity of the pneumococcal capsular polysaccharides by Avery and his colleagues came at a time when it was generally considered that such specificity was attributable only to proteins. Following the lead given by the work of Avery, specific serological reactivity was later shown to be due to polysaccharides in other bacterial groups – the tubercle bacillus, Friedlander's bacillus, *Haemophilus influenzae, Salmonella* and other organisms. Avery's group showed that there was serological cross-reactivity between polysaccharides from type II pneumoccoci, from a strain of Friedlander's bacillus (Avery, Heidelberger & Goebel, 1925) and from gum arabic (Heidelberger, Avery & Goebel, 1929). These cross-reactions were in part explained by the studies of Avery and Goebel, published in eight papers between 1929 and 1934, on conjugated carbohydrate-proteins. These showed that the fundamental specificity of sugar residues was dependent on configuration, size and nature. This work provided one of the foundation stones for the science of Immunochemistry.

In addition to the work I have touched upon, many other papers dealing with bacterial growth and lysis and C reactive protein came from Avery's laboratory. Many of these papers were published by Avery's colleagues alone, for he would never allow his name on a paper unless he had personally done a considerable proportion of the work involved. For example, of 61 papers from his department from 1930 to 1934 inclusive, only 14 bore Avery's name. For at least fifteen years Avery supervised and guided the work of Lancefield on the antigenic structure of streptococci. He was co-author of only the first two papers, one of which in 1919 demonstrated that there were different immunological types of haemolytic streptococci (Dochez, Avery & Lancefield, 1919).

The quality of Avery's scientific contributions was recognized by his election as a Foreign Member of the Royal Society, just before the paper on DNA and the transforming principle was published. The award of the Copley medal in 1945, the highest distinction the Royal Society could confer, was made in recognition of the importance of that discovery.

Burnet records of his meeting with Avery in 1943: 'Avery was an oldish man then, beginning to live a little in the past, and happy to relate to interested visitors how his work with the pneumococcus had reached this climax'. Burnet did not realize that for many years Avery had been putting on this carefully rehearsed performance for the benefit of

visitors and new members of staff. It was an exciting and absorbing story which lost nothing in the telling. For new assistants it was valuable as the background into which they could fit according to their inclinations or special skills.

For an insight into Avery's personality it is worth reading part of the speech he made when presenting the Kober Medal of the American Association of Physicians to Dochez in 1949. This was quoted by MacLeod when he wrote Avery's obituary (MacLeod, 1957). 'Throughout his studies there is unique continuity of thought centering in the dominant problem of acute respiratory diseases. The results of his work are not random products of chance observations. They are the fruits of years of wise reflection, objective thinking and thoughtful experimentation. I have never seen his laboratory desk piled high with Petri dishes and bristling with test tubes, like a forest wherein the trail ends and the searcher becomes lost in dense thickets of confused thought. I have never seen him so busy taking something out of one tube and putting it into another there was no time to think of why he was doing it or what he was actually looking for. I have never known him to engage in purposeless rivalries or competitive research. But often I have seen him sit calmly by, lost in thought while all around him others with great show of activity were flitting about like particles in Brownian motion; then, I have watched him rouse himself, saunter to his desk, assemble a few pipettes, borrow a few tubes of media, perhaps a jar of mice, and then do a simple experiment which answered the very question he had been thinking about, when others thought he had been idling in aimless leisure.' MacLeod adds that this paragraph epitomizes Avery's own approach to investigation and his philosophy of the 'true inwardness of research'. Another colleague, Dubos, wrote in 1956: 'That he was not made a Nobel Laureate remains to this day a matter of painful surprise in many scientific circles, since all his discoveries had an obvious quality of perfection and finality, immediate useful application, and great influence in moulding the activities of other investigators. One might hope that the Nobel Academy will some day acknowledge this oversight and publicly recognize as once the Académie Française did for Molière

> Rien ne manquait à sa gloire
> Il manquait à la notre'.

If I have seemed to devote an unduly large part of this address to Avery and his work, it is because I shared with Colin MacLeod a great regard and respect for Avery as a man and as a scientist. And I feel that Fred Griffith would not have begrudged this small tribute to one who had brought to such a successful conclusion the story which he so dramatically began.

REFERENCES

ALLOWAY, J. L. (1932). The transformation *in vitro* of R pneumococci into S forms of different specific types by the use of filtered pneumococcus extracts. *Journal of Experimental Medicine* **55**, 91–99.

ALLOWAY, J. L. (1933). Further observations on the use of pneumococcus extracts in effecting transformation of type *in vitro*. *Journal of Experimental Medicine* **57**, 265–278.

AUSTRIAN, R. & MACLEOD, C. M. (1949*a*). A type specific protein from pneumococcus. *Journal of Experimental Medicine* **89**, 439–450.

AUSTRIAN, R. & MACLEOD, C. M. (1949*b*). Acquisition of M protein by pneumococci through transformation reactions. *Journal of Experimental Medicine* **89**, 451–460.

AVERY, O. T., CHICKERING, H. T., COLE, R. & DOCHEZ, A. R. (1917). *Acute Lobar Pneumonia: Prevention and Serum Treatment*. Monograph no. 7. New York: Rockefeller Institute for Medical Research.

AVERY, O. T. & DUBOS, R. (1931). Protective action of a specific enzyme against type III pneumococcus infection in mice. *Journal of Experimental Medicine* **54**, 73–90.

AVERY, O. T. & GOEBEL, W. F. (1933). Chemoimmunological studies on the soluble specific substance of pneumococcus. I. The isolation and properties of the acetyl polysaccharide of pneumococcus type 1. *Journal of Experimental Medicine* **58**, 731–755.

AVERY, O. T. & HEIDELBERGER, M. (1923, 1925). Immunological relationships of cell constituents of pneumococcus. *Journal of Experimental Medicine* **38**, 81–85; **42**, 367–376.

AVERY, O. T., HEIDELBERGER, M. & GOEBEL, W. F. (1925). The soluble specific substance of Friedländer's bacillus. II. Chemical and immunological relationships of pneumococcus type II and of a strain of Friedländer's bacillus. *Journal of Experimental Medicine* **42**, 709–725.

AVERY, O. T., MACLEOD, C. M. & MCCARTY, M. (1944). Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from type III pneumococcus. *Journal of Experimental Medicine* **79**, 137–157.

BRACO, R. M., KRAUSS, M. R., ROE, A. S. & MACLEOD, C. M. (1957). Transformation reactions between pneumococcus and three strains of streptococci. *Journal of Experimental Medicine* **106**, 247–259.

BURNET, F. M. (1968). *Changing Patterns. An Atypical Autobiography*, p. 81. London: Heinemann.

DALE, H. (1946). Address of the President. *Proceedings of the Royal Society* B **133**, 123–139.

DAWSON, M. H. & SIA, R. H. P. (1931). *In vitro* transformation of pneumococcal types. I. A technique for inducing transformation of pneumococcal types *in vitro*. *Journal of Experimental Medicine* **54**, 681–699.

DAWSON, M. H. & WARBASSE, A. (1931). Further observations on the transformation of type-specific pneumococci by *in vitro* procedures. *Proceedings of the Society of Experimental Biology and Medicine* **29**, 149–151.

DOCHEZ, A. R. & AVERY, O. T. (1917). The elaboration of specific soluble substance by pneumococci during growth. *Journal of Experimental Medicine* **26**, 477–493.

DOCHEZ, A. R., AVERY, O. T. & LANCEFIELD, R. C. (1919). Studies on the biology of streptococcus. I. Antigenic relationships between strains of *Streptococcus haemolyticus*. *Journal of Experimental Medicine* **30**, 179–213.

DOCHEZ, A. R. & GILLESPIE, L. J. (1913). A biologic classification of pneumococcus by means of immunity reactions. *Journal of the American Medical Association* **61**, 727–732.

DUBOS, R. (1935). Studies of the mechanism of production of a specific bacterial enzyme which decomposes the capsular polysaccharide of type III pneumococcus. *Journal of Experimental Medicine* **62**, 259–269.

DUBOS, R. (1956). Oswald Theodore Avery. *Biographical Memoirs of Fellows of the Royal Society* **2**, 35–48.

DUBOS, R. & AVERY, O. T. (1931). Decomposition of the capsular polysaccharide of pneumococcus type III by a bacterial enzyme. *Journal of Experimental Medicine* **34**, 51–71.

FRANCIS, T., JUN. (1934). Antigenic action of the specific polysaccharide of pneumococcus type I in man. *Proceedings of the Society of Experimental Biology and Medicine* **31**, 493–495.

FRANCIS, T., JUN., TERRELL, E. E., DUBOS, R. & AVERY, O. T. (1934). Experimental type III pneumococcus pneumonia in monkeys. II. Treatment with an enzyme which decomposes the specific capsular polysaccharide of pneumococcus type III. *Journal of Experimental Medicine* **59**, 641–668.

FRANCIS, T., JUN. & TILLETT, W. S. (1930). Cutaneous reactions in pneumonia. The development of antibodies following the intradermal infection of type specific polysaccharide. *Journal of Experimental Medicine* **52**, 573–585.

GOODNER, K., DUBOS, R. & AVERY, O. T. (1932). The action of a specific enzyme upon the dermal infection of rabbits with type III pneumococcus. *Journal of Experimental Medicine* **55**, 393–404.

GRIFFITH, F. (1922). Types of pneumococci obtained from cases of lobar pneumonia. In *Reports on Public Health and Medical Subjects*, no. 13, pp. 20–45. London: H.M. Stationery Office.

GRIFFITH, F. (1928). The significance of pneumococcal types. *Journal of Hygiene* **27**, 113–159.

HAYES, W. (1966). Genetic transformation: A retrospective appreciation. *Journal of General Microbiology* **45**, 385–397.

HEIDELBERGER, M. & AVERY, O. T. (1923). The soluble specific substance of pneumococcus. *Journal of Experimental Medicine* **38**, 73–79.

HEIDELBERGER, M. & AVERY, O. T. (1924). The soluble specific substance of pneumococcus. Second Paper. *Journal of Experimental Medicine* **40**, 301–316.

HEIDELBERGER, M., AVERY, O. T. & GOEBEL, W. F. (1929). A 'soluble specific substance' derived from gum arabic. *Journal of Experimental Medicine* **49**, 847–857.

HEIDELBERGER, M., GOEBEL, W. F. & AVERY, O. T. (1925). The soluble specific substance of pneumococcus. Third Paper. *Journal of Experimental Medicine* **42**, 727–745.

HERSHEY, A. D. & CHASE, M. (1953). Independent functions of viral protein and nucleic acid in the growth of bacteriophage. *Journal of General Physiology* **36**, 39–56.

KRAUSS, M. R. & MACLEOD, C. M. (1963). Intraspecies and interspecies transformation reactions in pneumococcus and streptococcus. *Journal of General Physiology* **46**, 1141–1150.

MCCARTY, M. & AVERY, O. T. (1946). Studies on the chemical nature of the substance inducing transformation of pneumococcal types. II. Effect of desoxyribonucleose on the biological activity of the transforming substance. III. An improved method for the isolation of the transforming substance and its application to pneumococcus types II, III and VI. *Journal of Experimental Medicine* **83**, 89–96; 97–104.

MACLEOD, C. (1957). Oswald Theodore Avery. *Journal of General Microbiology* **17**, 539–549.

MACLEOD, C. & KRAUSS, M. R. (1950). Relation of virulence of pneumococcal strains for mice to the quantity of capsular polysaccharide formed *in vitro*. *Journal of Experimental Medicine* **92**, 1–9.

NEUFELD, F. & HÄNDEL, L. (1909). Über Herstellung und Prüfung von Antipneumokokken-Serum und über die Aussichten einer spezifischen Behandlung der Pneumonie. *Zeitschrift für Immunitätsforschung* **3**, 159–171.

NEUFELD, F. & HÄNDEL, L. (1912). Zur Frage der Serumtherapie der Pneumonie und der Wertbestimmung des Pneumokokken-Serums. *Berliner Klinische Wochenschrift* **49**, 680–683.

NEUFELD, F. & LEVINTHAL, W. (1928). Beiträge zur Variabilität der Pneumokokken. *Zeitschrift für Immunitätsforschung* **55**, 324–340.

POLLOCK, M. R. (1970). The discovery of DNA: an ironic tale of chance, prejudice and insight. *Journal of General Microbiology* **63**, 1–20.

SCHIEMANN, O. & CASPAR, W. (1927). Sind die spezifisch präcipitablen Substanzen der 3 Pneumokokken-Typen Haptene? *Zeitschrift für Hygiene und Infektionskrankheiten* **108**, 220–257.

SCHIEMANN, O., LOEWENTHAL, H. & HACKENTHAL, H. (1931). Über die immunisierende und schockerzeugende Wirkung von C-haltigen Fractionen gereinigter C-Substanz und Lipoiden aus Pneumokokken. *Zeitschrift für Hygiene und Infektionskrankheiten* **112**, 315–331.

SIA, R. H. P. & DAWSON, M. H. (1931). *In vitro* transformation of pneumococcal types II. The nature of the factor responsible for the transformation of pneumococcal types. *Journal of Experimental Medicine* **54**, 701–710.

STRYKER, L. M. (1916). Variations in the pneumococcus induced by growth in immune serum. *Journal of Experimental Medicine* **24**, 49–68.

TAYLOR, H. E. (1949). Additive effects of certain transforming agents from some variants of pneumococcus. *Journal of Experimental Medicine* **89**, 399–424.

WRIGHT, H. D. (1941). Obituary on W. M. Scott and F. Griffith. *Lancet* i, 588–589.

WYATT, H. V. (1972). When does information become knowledge? *Nature, London* **235**, 86–89.