A Retrospective Look: How We Identified the Pneumococcal Transforming Substance as DNA
By Maclyn McCarty

The day-to-day activities of laboratory research are usually not recorded in a fashion that makes it possible years later to recapture the full flavor of a project or indeed to extract detailed information about the rationale that led to each step along the way. This is certainly true of the search for the chemical identity of the pneumococcal transforming substance. Among the questions about the work that I have found myself unable to deal with satisfactorily in the past is a central one: When and how did we first become convinced that the active material was almost certainly DNA? There was clearly no flash of sudden revelation, no moment at which we could shout “Eureka.” I can recall a long period when the work was carried out in an atmosphere of only half-suppressed excitement as the scent of success became unmistakable, but my recollections of this period do not include a tidy picture of the set of experimental findings that led to the excitement in the first place. The present occasion seemed like an appropriate time to revisit the old laboratory notes and devote some time and effort to determining whether it was possible to reconstruct the order of events and our interpretation of them. While I can claim to be no more than capable, but my recollections of this period do not include a tidy view of the experiment. This may come as a surprise to those who picture the work as progressing continuously through the 1930s, after the studies of J. Lionel Alloway in Avery’s laboratory on the successful use of cell-free extracts in transformation. The second and last of Alloway’s papers appeared in the February 1933 issue of The Journal of Experimental Medicine. It is true that the subject was pursued for the next few years (first by Edward S. Rogers, who was in the laboratory from 1932 to 1934, and then more extensively by Colin MacLeod when he arrived in 1934) but there was a hiatus of sorts in the last years of the decade. Colin had tackled many aspects of the problem in an effort to make the phenomenon more consistent and reliable so that it could be subjected to further analysis. This work involved the preparation of active extracts; the definition of susceptible R strains of pneumococci; the nature of the serum factor required in the system; the occurrence of enzymes in the pneumococcus and other materials that destroy the transforming substance; and so on. Those studies were not published but were described in some detail in the annual spring Report to the Board of Scientific Directors from the laboratory in 1935 and again in 1937. However, for the next three years, in the annual reports of 1938 through 1940, the subject was not mentioned. As a result of frustrations generated by the vicissitudes and uncertain reproducibility of these experiments, this particular research effort had apparently given way almost entirely to more immediately successful enterprises. These included, among others, MacLeod’s work on the sulfonamides, on which he published several papers in 1939–1941, and the definitive studies with Avery on another important topic, one that had its origin in the laboratory. This dealt with the substance that appears in human blood during the acute phase of many conditions and came to be known as C-reactive protein.

Regardless of the details of their research during this period, this sequence of events explains the fact that in the famous letter to his brother Roy, written in May 1943, Avery introduced his description of the ongoing research on transformation by saying: “For the past two years, first with MacLeod and now with Dr. McCarty I have been trying to find out what is the chemical nature of the substance in the bacterial extract which induces the specific change.” I am suggesting that he shortened the period slightly, since it was a little over 2.5 years from the resumption of this research at the time the letter was written. The important point is that he did not say “For the past ten years.” It is my belief that Avery and MacLeod had agreed in the spring of 1940 that after the summer holidays they would together return to the study of the transforming substance and devote full effort to it. The first page in the loose-leaf laboratory notebook, dated October 22, 1940, has the description of an experiment written by MacLeod but bearing a heading on the sheet in Avery’s hand. The heading says “Exp. 1 (T.P.),” “T.P.” being the laboratory shorthand for transforming principle (Fig. 1). This was thus looked on as a new beginning. The experiment itself epitomizes past and persistent difficulties and involves a test of the effect of fluoride in the protection of the transforming principle from destruction during lysis of pneumococci.

The nature of the search involved is deceptively simple on paper. It can be illustrated by the kind of rough diagram that was commonly used in informal discussions but never published (Fig. 2). In the model system selected, cell-free extracts were prepared from mass cultures of type III pneumococci, an organism that produces a capsule of macromolecular polysaccharide, indicated in the figure by the stippled area around
the diplococcus. The polysaccharide is composed of a single repeating disaccharide unit, glucuronido-1-3-glucose. When the extract is added under appropriate cultural conditions to a medium in which an unencapsulated R strain of type II pneumococcus is growing, it leads to the emergence of organisms that produce the type III polysaccharide. Since the type IIIR organisms originally produced a chemically and serologically quite different polysaccharide, one that has rhamnose as its major constituent, it would appear that the type IIIR strain has been induced to synthesize an entirely new product, which it continues to do indefinitely on subculture. The specificity is determined by the extract: an extract from type I pneumococci induces the appearance of type I transformed cells; a type VI extract induces type VI, and so on. Thus a specific and heritable change had been induced.

The aim of the research was simply to identify the substance in the extracts responsible for this striking biological effect. The search began without preconceived notions as to the possible answer, and was thus prepared to follow any course dictated by the facts that were uncovered. The techniques available for the separation and analysis of biological macromolecules were quite primitive by today's standards, and progress toward this well-defined goal was certainly not rapid. When Avery and Macleod returned to this task in October 1940, they prepared type III pneumococcal transforming...
extracts by a slight modification of the procedure originally described by Alloway. This involved rapid lysis of a heavy suspension of type III pneumococci by the addition of the bile salt, sodium deoxycholate. To minimize destruction of transforming activity by the autolytic enzymes of the organism, lysis was carried out at 0°C in an ice bath; and as soon as it appeared complete (usually in about 15–30 minutes), the lysate was rapidly brought to 65°C and held there for 30 minutes to inactivate the offending enzymes. This procedure had the merit of releasing essentially all of the soluble components of the pneumococcal cells but the disadvantage of being highly variable and unpredictable in the biological activity of the product in the transforming system. This had been one of the major stumbling blocks to progress of the research in the mid-1930s, and it continued to plague these renewed efforts.

An example of this is provided by an experiment of November 5, 1940 in which the first three extracts prepared that fall were compared for transforming activity (Fig. 3). Extracts 1 and 2 were preliminary runs made from 3-liter batches of pneumococcal culture, while extract 3 used the same procedure scaled up in size to involve organisms from 36 liters of culture. In all cases fluoride had been used in an effort to inhibit inactivation of the transforming substance during lysis. In the table, "R" indicates no transformation and "SIII 100%" indicates that only transformed colonies were found on the culture plate. The total lack of activity of extract 3, with no evidence of transformation even when 1 ml of the undiluted material was used, confirmed an earlier titration. It is worth noting that even extracts 1 and 2 were feebly by comparison with results obtained later and must have represented only a minute fraction of the transforming activity originally present in the cells.

During the remainder of the fall and winter, Avery and MacLeod persevered in trying to improve the situation, focusing primarily on attempts to limit further the enzymatic destruction of the transforming substance. At the same time, they explored a number of different approaches to the fractionation of active extracts, the most successful and revealing of which was the application of the chloroform method of Sevag to deproteinization of the material. Repeated shaking of the extracts with chloroform and amyl alcohol, followed by removal of insoluble material by centrifugation, reduced the amount of protein to levels no longer detectable by qualitative tests without affecting the transforming activity. This became a part of the purification process for all future extracts.

On January 28, 1941, in connection with a repeat experiment on the lack of effect of crystalline ribonuclease on transforming activity, an important step was taken toward getting on the right track. For the first time, the Dische diphenylamine reaction for deoxyribose was applied to the transforming extracts. There is no indication as to who had called their attention to this test, which had first been described a decade earlier. In any event, Ex 5/40, which was used in this ribonuclease experiment, gave a positive reaction. Colin wrote the following conclusion in recording the experiment (Fig. 4): "Thus it would appear as though these transforming extracts contain a little deoxyribosenucleic acid in addition to the large amount of ribosenucleic acid present."

It may seem strange to some that the occurrence of deox-
yribonucleic acid in pneumococci, or any other bacteria, would still be uncertain as late as 1941. However, the facts on the distribution of nucleic acids were not yet well defined. Indeed, we were at that time just beginning to emerge from a period when there were thought to be two general classes of nucleic acid in nature: plant nucleic acid, as typified by the yeast preparations; and animal nucleic acid, as typified by thymus and fish sperm preparations. The textbooks that had served as my introduction to biochemistry a few years earlier subscribed to this view, and one of these added the following statement, which illustrates the state of knowledge in the 1930s: "It is not known with certainty that they [the nucleic acids] ever occur in the cytoplasm."

While MacLeod expressed some caution in subsequent notes about accepting the diphenylamine reaction as wholly specific for deoxyribose, these doubts seem to have been dispelled and gradually the test came to be applied with some regularity to monitor fractionation procedures.

Another important step was taken in mid-March 1941 when they gave up the Alloway method of making extracts by lysis of pneumococci. The procedure was introduced of heat killing the pneumococci at 65°C immediately after harvesting the cells, followed by extracting the killed cells in saline containing deoxycholate at higher concentrations than that used in initiating the lysis of living cells. This procedure dearly reduced the efficiency of solubilizing the contents of the pneumococci, but this was more than offset by the advantage of inactivating the enzyme that attacked the transformed substance before the extraction process. As the technique was perfected, it became possible consistently to obtain crude extracts of higher potency than any that had been made by the old procedure.

The laboratory notes suggest some interesting byplay between Avery and MacLeod in adopting this new procedure and even a possibility that there was a difference of opinion about the promise of the approach. While most of the notes during this period were kept by MacLeod, the first experiment on the extraction of heat-killed cells and all of the subsequent data on this preparation and its testing, between the 11th and 19th of March, were recorded in Avery's hand. Fig. 5 shows the initial page of this experiment. This was a trial run on a 2-liter batch of organisms, and on March 18 a 40-liter lot was grown and organisms divided into two equal portions. Avery extracted his half after heat killing the cells, keeping the notes on the preparation himself, and MacLeod handled the other half by the usual deoxycholate lysis followed by heating. The results of this experiment were rendered somewhat inconclusive due to the recurring difficulties with the transforming system in which the extracts were titrated. However, Avery's extract was at least as active as MacLeod's and had much less serologically active material by precipitin test. It must have settled the issue in any event, since MacLeod next prepared an extract from a 50-liter batch using the extraction of heat-killed cells, and all subsequent extracts for the next few years used this approach.

The spring and early summer of 1941 were devoted to collecting material by this procedure and using it in attempts to develop effective fractionation procedures, chiefly by means of alcohol and calcium precipitation. It had been observed since Alloway's day that the pneumococcal extracts tended to be quite viscous and to yield variable amounts of stringy or fibrous precipitate on the addition of alcohol. The separation of this type of precipitate from the more voluminous flocculent precipitates produced by alcohol naturally became one object of these fractionation experiments. Here a red her ring was introduced in the form of the type III capsular polysaccharide, a major constituent of the extracts. It became evident that the polysaccharide was separating out in the form of fibrous strands of precipitate at concentrations of alcohol below 50%, and for a period all material precipitating in this manner appears to have been equated with polysaccharide.

The results of fractionation with alcohol were rendered variable by fluctuations in the concentration of materials in the extracts, and while transforming activity tended to go along with the polysaccharide in some fractionations, there was a considerable degree of separation of the two in certain other experiments. In early July 1941, just as he was departing

Figure 5. Avery's notes on the first use of heat-killed cells for the extraction of T.P.
to assume the chair of microbiology at New York University, MacLeod summarized the current status of the preparative methods and included the final comment: "In this process of purification there has been a great loss of activity. It may be necessary to increase the concentration of SSS [type III polysaccharide] to improve the results."

While I am not certain that this comment refers to it, the idea had arisen that the presence of type III polysaccharide in the transforming system might be necessary to serve as a template or primer for the de novo synthesis of polysaccharide by the transformed pneumococci. This explains why one of my first projects after joining the laboratory in September 1941 was to return to a problem that had been looked at much earlier by both Rogers and MacLeod. This dealt with the effect on transforming extracts of the Dubos enzyme, derived from a soil bacillus, that specifically hydrolyzes the type III polysaccharide, yielding as an end product of complete hydrolysis the disaccharide repeating unit. This project served to acquaint me firsthand with the important store of knowledge of the laboratory on the pneumococcal polysaccharides as well as with the exasperating variability in the behavior of the transforming system.

Because of this variability, it was not until the end of November that I succeeded in completing an unequivocal experiment showing that enzymatic destruction of type III polysaccharide, so that it was no longer serologically detectable (i.e., <0.2 μg/ml), had no effect on the transforming activity of an extract (Fig. 6). In this experiment, after dialysis to remove enzymatic split products, the material was tested for serological activity and titered in the transforming system. The titration shown here indicates that there was no difference in transforming activity between the enzyme-treated sample and the controls.

As a result of this reconfirmation that the polysaccharide

![Figure 6](image-url)

**Figure 6.** Experiment demonstrating that type III polysaccharide is not required in the transforming system.
is not needed in transformation, it seemed desirable to devise preparative methods that would provide us with purified extracts devoid of type III polysaccharide. The first step was to reduce the amount of polysaccharide initially present in the crude extract so that removal of the residuum from preparative lots would not overwhelm our limited supplies of the SIII enzyme. One possible way to achieve this was to eliminate the customary procedure of adding excess glucose for the last 2 hours of cultivation of the type III pneumococci to enhance growth and the yield of organisms for extraction. This procedure was effective for its purpose but also greatly enhanced the production of capsular polysaccharide.

In an experiment stimulated by the results with the SIII enzyme and carried out just a few days before Pearl Harbor, a 50-liter batch of organisms was grown without the addition of extra glucose and the cells extracted as usual after heat killing. Not only did the extract have less polysaccharide than previous lots, but it turned out by chance to be the most potent extract that had yet been obtained, clearly encouraging further efforts in this direction (Fig. 7). The titration shows that 0.3 ml of a 1:10,000 dilution of the extract induced transformation. (Parenthetically, the final comment on the page reveals that I experienced my own Pearl Harbor by breaking the flask and losing this potent preparation.)

Subsequent experiments revealed that it was advantageous to wash the heat-killed cells repeatedly in saline before deoxycholate extraction, thus further reducing not only the polysaccharide but also the ribonucleic acid content of the preparation without comparable loss of transforming activity. By

| Tube | Material | Amount | Turbidity | Transformation |
|------|----------|--------|-----------|----------------|
| 1    | a.       | Est. 14 | 0.5       | +             | SIII = 30% |
|      | b.       | 1:10   | 0.3       | +             | SII = 50% |
|      | c.       | 0.1    | +         | SII = 50%     |
| 2    | a.       | 1:100  | 0.5       | +             | SII = 90% |
|      | b.       | 0.3    | +         | SII = 40%     |
|      | c.       | 0.1    | +         | SII = 90%     |
| 3    | a.       | 1:1000 | 0.5       | +             | SII = 60% |
|      | b.       | 0.3    | +         | SII = 40%     |
|      | c.       | 0.1    | +         | SII = 90%     |
| 4    | a.       | 1:10,000 | 0.5 | + | SII = 50% |
|      | b.       | 0.3    | +         | SII = 50%     |
|      | c.       | 0.1    | -         | -              |

Figure 7. Titration of activity of preparation with reduced polysaccharide content.
the early months of 1942 we were able to make preparations that were devoid of protein, as indicated by the biuret test, and were also rendered essentially free of type III polysaccharide with the use of the Dubos SIII enzyme. It became obvious that preparations of this kind still yielded viscous solutions and contained material that formed fibrous precipitates on the addition of alcohol, establishing that the polysaccharide was not solely responsible for these characteristics.

Some experiments indicated that the fibrous alcohol precipitates carried most of the diphenylamine-reactive material and most of the transforming activity of the extract, although the separations were not always so sharp as could be desired. It took a while to learn the optimum conditions for this precipitation, since the results depended on a variety of factors, such as the concentration of the material and the manner in which the alcohol was added. One of the early successful experiments of this type, carried out on January 27, 1942, indicates that the fractionation of transforming activity by this technique could be quite effective (Fig. 8).

Our interpretation of these findings was assisted by the availability at about this time of authentic preparations of mammalian DNA from Alfred Mirsky. He had provided us with material isolated from thymus, spleen, and sperm by his elegant procedure based on the differential solubility of nucleohistone in salt solutions. These DNAs were supplied as fibrous alcohol precipitates that had been dried with ether,

| Extract #26 | Automated Precipitation (continued) |
|-------------|------------------------------------|
| 1/27/42     |                                    |
| Fraction A  | precipitate glycine and equimolar 50% deionized water. Due to lack of electrolyte 10 μl. 10% sucrose added with fraction A more precipitant and more strong. Preparated 2 hours and centrifuged. Precipitate taken up in 5 ml saline + 5 ml 0.05 M Tris buffer pH 7.6. |
| Fraction B  | KCl-precipitate recovered by centrifuging. Taken up in 5 ml saline + 5 ml 0.05 M Tris buffer pH 7.6. |

### Transforming Test in Fractions

|       | CA  | Neo | +4C | +10% Boehr |
|-------|-----|-----|-----|------------|
| Fraction A | 0.05 | + | + | + |
| Fraction B | 0.5  | + | + | + |

### Table

| Tube | Material                      | Dilution | Protein | Nucleic Acid | Activity |
|------|-------------------------------|----------|---------|--------------|----------|
| 1 a  | Fraction A                    | 1:10     | 0.5     | +            | S[4]     |
|      |                               | 1:10     | 0.3     | +            | S[4]     |
|      |                               | 1:10     | 0.1     | +            | S[4]     |
| 2 a  | [Ppt after 1:2]               | 1:100    | 0.5     | +            | S[4]     |
|      |                                | 1:100    | 0.3     | +            | S[4]     |
|      | [Ppt after 1:2]               | 1:100    | 0.3     | +            | S[4]     |
| 3 a  |                               | 1:1000   | 0.3     | +            | S[4]     |
|      | [Ppt after 1:2]               | 1:1000   | 0.3     | +            | S[4]     |
| 4 a  |                               | 1:10000  | 0.3     | +            | S[4]     |
|      | [Ppt after 1:2]               | 1:10000  | 0.3     | +            | S[4]     |
| 5 a  |                               | 1:10     | 0.5     | +            | S[4]     |
|      | [Ppt after 1:2]               | 1:10     | 0.5     | +            | S[4]     |
| 6 a  |                               | 1:10     | 0.5     | +            | S[4]     |
|      | [Ppt after 1:2]               | 1:10     | 0.3     | +            | S[4]     |
| 7 a  |                               | 1:100    | 0.3     | +            | S[4]     |

Figure 8. Fractionation experiment suggesting the transforming activity resides in the fibrous alcohol precipitate.
and when they were dissolved in saline gave highly viscous solutions. They served to acquaint us with the properties of native DNA. The similarity to the active transforming material that was precipitated by alcohol in fibrous form from our preparations was indeed striking.

Thus, by the spring of 1942 our attention had clearly become focused on the possibility that DNA was the substance responsible for transforming activity. At that point we obtained experimental evidence of a quite different sort that provided independent support for this view. Alexander Rothen was then working with an ultracentrifuge, the equipment filling a sizable room in the basement of what is now called Flexner Hall, with most of the space taken up by the schlieren optical system. He undertook to examine some of our material analytically in a sector-shaped cell that was divided into two compartments by a perforated partition about two-thirds of the way down the cell.

In the first experiment the material was spun at 50,000

![Table Image]

Figure 9. Analytical ultracentrifuge analysis of transforming extract.
rpm until the fastest moving visible boundary was well down in the lower compartment. The material was then removed from the two compartments separately, precipitated in alcohol for sterilization, reconstituted in saline, and titrated for transforming activity. The results (Fig. 9) were quite clear in indicating that the transforming substance was concentrated in the lower chamber, and although we were dealing with small volumes of material it was possible to carry out a few chemical tests showing that this was also true of the DNA. This evidence that the active material was of high molecular weight was confirmed in several additional analytical runs in which the boundary was moved to different levels of the cell.

We then proceeded to use the concentration head of the ultracentrifuge on a pool of transforming extracts that had been prepared late in 1941 and stored after freeze-drying. After resolubilizing in saline, material was distributed in plastic tubes for centrifugation. Centrifugation at 30,000 rpm for 4-6 hours sufficed to concentrate the transforming activity in the lower 1 ml of the 6 ml in the tubes. Fig. 10 shows the titration of one of the tubes, indicating that 95% or more of the activity was in the lower fraction. Indeed, in tubes spun for 6 hours there was a well-defined pellet at the bottom, gelatinous and translucent in appearance, that could easily be separated from the supernatant fluid. This pellet proved to contain 95-99% of the transforming activity and on chemical and serological analysis the only known constituent of the extract that was concentrated in the pellet was the diphenylamine-reactive material and thus presumably the DNA.

By the summer of 1942 there was thus more than one kind of evidence implicating DNA as the substance responsible for transforming activity. These results stimulated another look at the various enzymes that had been tested for their effect on the transforming substance. These included, in addition to certain crystalline enzymes, crude preparations of

![Figure 10. Preparative ultracentrifuge experiment.](image-url)
bone, kidney, and intestinal phosphatase, a purified preparation of pancreatic lipase, pneumococcal autolysates, and mammalian sera. They were compared under various conditions for their effect on transforming activity and their ability to depolymerize authentic DNA. Here again the results were unequivocal, and only those preparations that clearly acted on DNA were able to inactivate the transforming substance. While only a correlation, the findings provided enough support for what now seemed the most likely possibility to lead us on to a final major step, that of attempting to prepare highly purified preparations of pneumococcal DNA.

The plan adopted was first to perfect our purification process and then to prepare several lots that could be subjected to detailed analysis. The latter was to include elementary analysis, semiquantitative assay by chemical and immunological techniques to detect contaminating substances, enzymatic analysis, ultracentrifugal and electrophoretic studies, and of course quantitative titration of transforming activity. The purification procedures devised were based on the experience gained in preceding years and concluded with several precipitations for what now seemed the most likely possibility to lead us on to a final major step, that of attempting to prepare highly purified preparations of pneumococcal DNA.

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The first of these purified products was available for analysis in October 1942, and in the ensuing months three other preparations, each from organisms obtained from 200-300 liters of culture, were completed and studied in detail. By the time of Avery's letter to his brother in May 1943 most of the work had been done, and the "new batch" to which he refers to get "further evidence of purity and homogeneity by use of ultracentrifuge and electrophoresis" was already in its early stages of preparation. This was the final lot before writing up the work. By comparison with products isolated from mammalian sources, our preparations appeared to be of good quality DNA, highly active in transformation, and all of the analytical data were consistent with the conclusion that the primary constituent of the material was responsible for the biological activity.

During this period I am afraid that both Colin and I became increasingly impatient with Avery's caution, even though we were not unaware of the importance of being sure of our ground. We were just young enough to become convinced more readily. Avery expressed his doubts repeatedly in his letter to Roy and they were also obvious on almost a daily basis in the laboratory. The three of us made our pilgrimage to the Rockefeller Institute laboratories at Princeton at about this time, my unverifiable recollection is that it was in April 1943, to consult John Northrop and Wendell Stanley. Both of these workers had experienced skeptical responses to their reports attributing biological activity to a crystalline product: Northrop with pepsin and Stanley with tobacco mosaic virus. They of course were not able to provide us with a magic formula for eliminating the possibility that a contaminating substance was responsible for the transforming activity of pneumococcal DNA, and their advice was, in essence, "you just have to do the best you can."

It was on the train on the way back from Princeton that Colin said to Avery: "What else do you want, Fess? What more evidence do you need?" To the best of my recollection, Avery gave no specific answer, but I think that the "else" that he would have liked to have had was a purified DNase to try on the transforming DNA. This is in keeping with his reliance on other occasions on information obtained by applying enzymatic tools to biological problems. The very existence of the Dubos SIII enzyme, which played so important a role in final purification of the transforming DNA, depended on his earlier conviction that such an enzyme would be of great value in establishing that the type-specific antigens of pneumococci were indeed polysaccharides rather than some contaminating substance. He had been exposed to this kind of skepticism before. I think that he knew that we could not wait to publish our results until we had a suitable DNase, since my efforts to prepare the enzyme were just beginning at this time.

I have found on occasion that it is assumed by some that data on inactivation of the transforming substance by a well-characterized DNase was included in the 1944 paper. It was actually two years later when this information appeared in print, and it was thus somewhat anticlimactic. It did, however, serve as a coup-de-grace to the notion to which a few had clung that a trace of protein in the DNA preparations accounted for their biological activity. In addition, work on the enzyme provided indirect evidence of another sort through the effect that the knowledge of the properties of DNase had on the method of preparation of transforming DNA. Pancreatic DNase was found to require magnesium or manganese ion for its activity, and it was then determined that the DNase present in pneumococci also depended on divalent cations. It thus became possible to return to the procedure of Alloway and to use lysis of pneumococci for the efficient production of increased yields of transforming DNA by the simple expedient of including chelators of the activating ions in the lytic system. All of the difficulties with this procedure over the years were solved by this one modification, based on learning something about the inactivating enzyme.

I will close by describing briefly the actual writing of the first paper, after the collection of data was completed. This began in the summer of 1943, with Avery working on drafts of the introduction and discussion at his customary summer retreat on Deer Isle, Maine, and I collecting protocols and preparing a draft of the experimental part in New York. In the fall we reserved a small, quiet room in the library where we repaired for many long hours to revise and polish the manuscript. Avery applied to our efforts the same stringent criteria for which he was justly famous in his review of the manuscripts of others. It was a great relief to me when we got the completed version for The Journal of Experimental Medicine into the hands of Peyton Rous on November 1st.