APPENDIXES

APPENDIX I

A Letter from Avery to His Brother Roy, Dated May 26, 1943

(This is part of a letter from Avery to his brother Dr. Roy C. Avery. The first pages of the handwritten text were written on May 13, 1943; they are not reproduced here because they deal with family affairs in relation to Avery's proposed move from New York to join his family in Nashville, Tennessee. In fact, the entire letter is an explanation of the postponement of the move. Avery had reached the [then] mandatory retirement age of 65 at The Rockefeller Institute for Medical Research and was to become Emeritus Member in July, 1943.

The second part of the letter, dated May 26, is here reproduced. Although it is commonly believed that it presents the first written record of the role of DNA as carrier of genetic information, this is not quite true. All the facts and hypotheses mentioned in the letter are reported at length in the annual report that was submitted to the Board of Scientific Directors in the early spring of 1943.

Along with much factual information, the letter contains many phrases that Avery commonly used in everyday conversations. For example, after describing some properties of the transforming substance he adds, "Sounds like a virus—may be a gene. But with mechanisms I am not now concerned—One step at a time—and the first is, what is the chemical nature of the transforming principle? Someone else can work out the rest. Of course, the problem bristles with implications. . . . It's lots of fun to blow bubbles—but it's wiser to prick them yourself before someone else tries to. . . . It's hazardous to go off half cocked—and embarrassing to have to retract later."

The letter was terminated "long after midnight" and Avery apologizes for its deficiencies. "I'm so tired and sleepy I'm afraid I have not made this very clear. . . . Forgive this rambling epistle." In reality, the letter is far from rambling. Its technical parts are largely taken from the annual report written some two months earlier and are presented with precision and clarity. Even when writing to his brother, the Professor could not avoid playing one of his Red Seal Records! He also ended the letter with Dickens' phrase that he loved to use in the laboratory: "God bless us, one and all.")

Dr. Gasser and Dr. Rivers have been very kind and have insisted on my staying on, providing me an ample budget and technical assistance to carry on the problem that I've been studying. I've not published anything about it—indeed have discussed it only with a few—because I'm not yet convinced that we have (as yet) sufficient evidence. However, I did talk to Ernest [Dr. Ernest Goodpasture, Vanderbilt University Medical School] about it in Washington and I hope he has told you—for I have intended telling you first of all. I felt he should know because it bears directly on my coming eventually to Nashville.

It is the problem of the transformation of pneumococcal types. You will recall that Griffith, in London, some 15 years ago described a technique whereby he could change one specific type into another specific type through the intermediate R form. For example: Type II → R → Type III. This he accomplished by injecting mice with a large amount of *heat killed* Type III cells together with a small inoculum of a *living R culture* derived from *Type II*. He noted that not infrequently the mice so treated died and from their heart blood he recovered living, encapsulated Type III pneumococci. This he could accomplish only by the use of mice. He failed to obtain transformation when the *same* bacterial mixture was incubated in broth. Griffith's original observations were repeated and confirmed both in our Lab and abroad by Neufeld, and others. Then you remember Dawson with us reproduced the phenomenon *in vitro* by adding a dash of anti-R serum to the broth cultures. Later Alloway used *filtered extracts* prepared from Type III cells and in the absence of formed elements and cellular debris induced the R cultures derived from Type II to become typical encapsulated III pneumococcus. This you may remember involved several and repeated transfers in serum broth – often as many as 5-6 – before the change occurred. But it did occur and once the reaction was induced, thereafter without further addition of the inducing extract, the organisms continued to produce the Type III capsules; that is the change was hereditary and transmissible in series in plain broth thereafter. 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 extracts which induces this specific change. The crude extract (Type III) is full of capsular polysaccharide, C (somatic) carbohydrate, nucleoproteins, free nucleic acids of both the yeast and thymus type, lipids and other cell constituents. Try to find in that complex mixture the active principle!! Try to isolate and chemically identify the particular substance that will by itself when brought into contact with the R cell derived from Type II cause it to elaborate Type III capsular polysaccharide, and to acquire all the aristocratic distinctions of the same specific type of cells as that from which the extract was prepared! Some job – and full of heartaches and heart breaks. But at last *perhaps* we have it. The active substance is *not* digested by crystalline trypsin or chymotrypsin – It does not lose activity when treated with crystalline Ribonuclease which specifically breaks down yeast nucleic acid. The Type III capsular polysaccharide can be removed by digestion with the specific Type III enzyme without loss of transforming activity of a potent extract. The lipids can be extracted from such extracts by alcohol and ether at −12° C without impairing biological activity. The extract can be de-proteinized by Sevag Method (shaking with chloroform and amyl alcohol) until protein free and biuret negative. When extracts treated and purified to this extent, but still containing traces of protein, lots of C carbohydrate and nucleic acids of both the yeast and thymus types are further fractionated by the dropwise addition of absolute ethyl alcohol, an interesting thing occurs. When alcohol reaches a concentration of about 9/10 volume there separates out a fibrous substance which on stirring the mixture wraps itself about the glass rod like thread on a spool – and the other impurities stay behind as granular precipitate. The fibrous material is redissolved and the process repeated several times – In short, the substance is highly reactive and on elementary analysis conforms *very* closely to the theoretical values of pure *desoxyribose nucleic acid* (thymus type). Who could have

guessed it? This type of nucleic acid has not to my knowledge been recognized in pneumococcus before—though it has been found in other bacteria.

Of a number of crude enzyme preparations from rabbit bone, swine kidney, dog intestinal mucosa, and *pneumococci*, and fresh blood serum of human, dog and rabbit, only those containing active depolymerase capable of breaking down known authentic samples of desoxyribose nucleic acid have been found to destroy the activity of our substance—indirect evidence but suggestive that the transforming principle as isolated may belong to this class of chemical substance. We have isolated highly purified substance of which as little as 0.02 of a *micro*gram is active in inducing transformation. In the reaction mixture (culture medium) this represents a dilution of 1 part in a hundred million—potent stuff that—and highly specific. This does not leave much room for impurities—but the evidence is not good enough yet. In dilution of 1:1000 the substance is highly *viscous* as an authentic preparation of desoxyribose nucleic acid derived from fish sperm. Preliminary studies with the ultracentrifuge indicate a molecular weight of approximately 500,000—a highly polymerized substance.

We are now planning to prepare new batch and get further evidence of purity and homogeneity by use of ultracentrifuge and electrophoresis. This will keep me here for a while longer. If things go well I hope to go up to Deer Isle, rest awhile—Come back refreshed and try to pick up loose ends in the problem and write up the work. If we are right, and of course that's not yet proven, then it means that nucleic acids are *not* merely structurally important but functionally active substances in determining the biochemical activities and specific characteristics of cells—and that by means of a known chemical substance it is possible to induce *predictable* and *hereditary* changes in cells. This is something that has long been the dream of geneticists. The mutations they induce by X ray and ultraviolet are always unpredictable, random, and chance changes. If we are proven to be right—and of course that's a big *if*—then it means that both the chemical nature of the *inducing stimulus* is known and the chemical structure of the *substance produced* is also known—the former being thymus nucleic acid—the latter Type III polysaccharide. And both are thereafter reduplicated in the daughter cells and after innumerable transfers and without further addition of the inducing agent, the same active and specific transforming substance can be recovered far in excess of the amount originally used to induce the reaction. Sounds like a virus—may be a gene. But with mechanisms I am not now concerned—One step at a time—and the first is, what is the chemical nature of the transforming principle? Someone else can work out the rest. Of course, the problem bristles with implications. It touches the biochemistry of the thymus type of nucleic acids which are known to constitute the major part of the chromosomes but have been thought to be alike regardless of origin and species. It touches genetics, enzyme chemistry, cell metabolism and carbohydrate synthesis, etc. today it takes a lot of well documented evidence to convince anyone that the sodium salt of desoxyribose nucleic acid, protein-free, could possibly be endowed with such biologically active and specific properties and this evidence we are now trying to get. It's lots of fun to blow bubbles—but it's wiser to prick them yourself before someone else tries to. So there's the story Roy—right or wrong it's been good fun and lots of work. This supplemented by war work and general supervision of other important problems in the Lab has kept me busy, as you can well

understand. Talk it over with Goodpasture but don't shout it around—until we're quite sure or at least as sure as present method permits. It's hazardous to go off half cocked—and embarrassing to have to retract later.

I'm so tired and sleepy I'm afraid I have not made this very clear. But I want you to know—and sure you will see that I cannot well leave this problem until we've got convincing evidence. Then I look forward and hope we may all be together—God and the war permitting—and living out our days in peace. What a lovely picture of dear Margaret. How is she and Cath—wish we could all meet in Deer Isle. I know Minnie has kept you all posted. Things go well with us despite this cruel war but Victory must come and I'm optimistic enough to look forward to happier days even if they are not perfect—We can take it—and still be happy.

Forgive this rambling epistle—with it goes my love and thought and hope of better things ahead—

"With heaps and heaps of love"
Affectionately and faithfully,
OTA

[A P.S. but not so designated]

If the Board in the Surgeon General's office meets at Camp Bragg as I think they may later on I shall take the opportunity of running over to Nashville for I want to talk over future plans and possibilities with you and Catherine. Do write if just a line—I want to know your reaction and don't hestitate to talk to Ernest—he knows it all and we talked it over very frankly.

Good night—it's long after mid-night and I have a busy day ahead. God bless us, one and all. Sleepy, well and happy—