Part if a letter from Dr.O.T.Avery to his brother Roy C.Avery, dated May 13,1943, just after his retirement from the Rockefeller Institute, when he was considering moving to Nashville,Tennessee.

"Both Dr. Gasser and Dr. Rivers have been very kind and have insisted on my staying on, providing me an ample budget and technical assistant to carry on the problem that I have been studying. I have not published anything about it indeed have discussed it only with a few because I am not yet convinced that we have sufficient evidence as yet.

It is the problem of the transformation of pneumococcal types. You will recall that Griffith in London, some fifteen 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. Griffiths original observations were repeated and confirmed both in our laboratory 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 culture. Later Alloway used filtered extracts prepared from Type III cells in the absence of formed elements and cellular debris induced the R culture derived from Type II to become a typical incapsulated III pneumococcus. This you may remember involved several and repeated transferrs in serum broth often as many as five or six 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 capsule that is to say the change was hereditary and transmissable 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 extract which induces this specific change. The crude extract Type III, is full of capsular polysaccharide, C (somatic) carbohydrate, nucleoproteins,free neculeas acids of both the yeast and thymus type, lipids and other cell constituents. Try to find in the complex mixtures 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 causes 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, full of headaches and heartbreaks. 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 polysaccharide can be removed by digestion with the specific Type III enzyme without loss of transforming activity of a potent extract. Lipids can be extracted from such extracts by alcohol and ether at a minus 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 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 seperates 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, this 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 neucleic acid has not ~~been~~ to my knowledge been recognized in pneumococcus before though it has been found in other bacteria.

Of a number of crude enzymes 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 γ of a microgram is active in inducing transformation. In the reaction mixture (culture medium) this represents a dilution of one 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 dilutions of one to a thousand the substance is highly viscous as are authentic preparations of desoxyribose neucleic acid derived from fish sperm. Preliminary studies with the ultra centrifuge indicate a molecular weight of approximately 500,000 - a highly polymerised substance.

We are now planning to prepare new batches and get further evidence of purity and homogeneity by use of the ultracentrifuge and electrophoresis. This will keep me here for awhile longer if things go well I hope to go up to Deer Isle rest awhile ~~rest a~~ 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 is not yet proven, then it means that neucleic acids are not merely structially 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 induced by ex-ray and ultra-violet are always unpredictable, random, amd chance changes if we prove to be right-and of course that is 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 form being thymus nuclecic acid, the latter Type III polysaccharides and both are ~~thereafter~~ reduplicated in the daughter cells and after innumerable transfers 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 step is what is the chemical nature of the transforming principle ? Some one else can work out therest. Of course the problem bristles with implications. It touches biochemistry of the thymus type of neucleic acids which are known to constitute the major part of chromosomes but have been thought to be alike regardless of origin and species. It touches genetics, enzymechemistry, cell metabolism and carbohydrate synthesis. But to-day it takes a lot of well documented evidence to convince anyone that the sodium salt of desoxyribose ~~neucleic~~ acid, protein free, could possibly be endowed with such biologically active and specific propoties and that is the evidence we are now trying to get. It is lots of fun to blow bubbles but it is wiser to prick them yourself before some one else tries to."

End of Page 12