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AVERY

Dear Josh,

Enclosed is a copy of the text of Avery's letter to his brother. Several years ago, I wrote a history of the discovery of transformation, which I never published, and had included the letter. I have xeroxed the two pages of my text, since we are having secretarial troubles right now. It's messy, and my apologies, but it is all there.

Boris enjoyed his evening with you and Esther very much, and communicated to me your sympathetic remarks. The only really distressing thing about our situation is that I have built up a splendid group here, and we are able to explore transformation as I could never do in the past. Some areas of research cannot go ahead at any reasonable rate when one is alone with a technician, or aided with a temporary associate.

announcing that the ~~active~~ capsular transforming principle was, in all probability, a desoxyribonucleate. The proof brought to bear on the identity of the factor was summarized by Avery in a letter written to his brother, also a medical bacteriologist, in 1943:

" The active substance is not digested by crystalline trypsin or chymotrypsin, it does not lose activity when treated with crystalline ribonulcease 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 minus 12° C without impairing biological activity. The extract can be deproteinized by t the Sevag method (shaking in 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 (somatic) carbohydrate, and nucleic acids of both yeast and thymus types are further fractionated by dropwise addition of absolute ethyl alcohol an interesting thing occurs. When alcohol reaches a concentration of about 9/10 volumes there separates out a fibrous substance which on stirring the mixture wraps itself about a glass rod like thread on a spool and the other impurities stay behind as a 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 desoxyribonucleic 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."

In the same letter, Avery stated that of a number of crude enzyme preparations from various sources, only those which contained a very active depolymerase were able to ~~inactivate~~ destroy the transforming factor. The purification and identification of this polymerase was to be the next stepping stone in the advance toward certitude of the identity of the transforming agent.

Finally, Avery concluded his letter in this fashion:

"If we are right, and of course that is not yet proven, then it means that nucleic acids are not merely structurally important, but functionally ~~important as well~~ active substances in determining biochemical activites 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 X-rays and ultra-violet are always unpredictable, random, and 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 nature of the substance produced is also known, the former being thymus nucleic acid, and the latter, Type III polysaccharide, and both are thereafter reduplicated in the daughter cells and after inumerable 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--maybe 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? Someone else can work out the rest. Of course the problem bristles with implications. It touches biochemistry of the thymus type of nucleic acids which are known to constitute the major part of chromosomes, but have been thought to be alike regardless of origin and of species. It touches genetics, enzyme chemistry, cell metabolism and carbohydrate synthesis. But today it takes a lot of documented evidence to convince anyone that the sodium salt of desoxyribonucleic acid, protein free, could possibly be endowed with such biologically active and specific properties, and that is the evidence we are now trying to get. Its lots of fun to blow bubbles but it is wiser to prick them yourself before someone else tries to."

On the basis of these results, a vast program of research could have been organized. In fact, what was projected was the continuation of the strategy which Avery had practised throughout his entire scientific life: that of eliminating the most important obstacle in the way of a generalization. This obstacle was, as he saw it, the doubt as to the chemical identity of the transforming principle, or TP, as it was now familiarly called. This doubt took two forms: one was the possibility that trace contaminants in the DNA were responsible for biological activity, and the other was the possibility that serum, necessary for successful ~~bioassay~~, was playing a role of primary importance in the reaction, forming a nucleoprotein, for example, which was the active material rather than the DNA itself.

With the purification of the enzyme desoxyribonuclease, achieved by McCarty in 1946, a new and powerful tool was made available for transformation studies. The enzyme proved to be activated by Mg^{++} and Mn^{++}. Minute amounts of desoxyribonuclease rapidly destroyed all activity of the purified DNA endowed with the Type III transforming activity, provided appropriate amounts of the activating ions were present. If they were absent, or withdrawn by a chelating agent, no inactivation of TP occurred. Although transforming activity of DNA was damaged before measureable changes in viscosity could be detected, the conditions under which inactivation occurred were identical with those under which depolymerization took place.